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Genetic variability and multi-trait selection of biomass and stalk juice for bioenergy in sorghum segregating populations

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Abstract. *Dare D, Trikoesoemaningtyas, Wirnas D, Soepandi D. 2024. Genetic variability and multi-trait selection of biomass and stalk juice for bioenergy in sorghum segregating populations. Biodiversitas 25: 4425-4437.* Sorghum (*Sorghum bicolor* (L.) Moench) is a promising crop for biofuel production due to its high sugar content in stem juice, which can be converted into bioethanol. However, the challenge for plant breeders developing bioenergy sorghum is creating superior genotypes with enhanced biomass-related traits. This study aimed to evaluate the genetic variability and multi-trait selection of biomass and stalk juice in sorghum segregating populations. A total of 320 F2 genotypes and six check genotypes were evaluated. Observed parameters included biomass-related traits and grain yield. Data analysis comprised gene action and genetic parameter estimation, Pearson correlation, path analysis, transgressive segregant estimation, multi-trait selection, cluster, and principal component analysis. The results showed high genetic variability for biomass-related traits, with coefficients of variation for fresh biomass and juice volume of 40.64% and 68.03%, respectively. Broad-sense heritability for these traits ranged from low (0%) to high (89.27) and was influenced by additive genes and complementary epistasis. All characters showed significant correlations with target traits, except for the Brix value. Direct selection for juice volume and fresh biomass weight can be achieved through stem weight. The transgressive index revealed high values, with transgressive segregants at 5.43% and 1.94%, respectively. Multi characters selection identified the 26 best genotypes for further testing.

Keywords: Bioenergy, biomass, genetic variability, juice yield, multi-trait selection, *Sorghum bicolor*

INTRODUCTION

The annual population growth drives higher energy demand. However, fossil-based energy sources are limited in availability. Therefore, environmental friendly energy sources alternative, such as biofuels are essential (Vitazek et al. 2018). Biofuels, primarily produced from biomass, are in increasing demand owing to their renewability and sustainability (Ruan et al. 2019). Sorghum (*Sorghum bicolor* (L.) Moench) has proven to be a significant source of biomass for biofuel production. It is chosen for bioenergy production not only for its high biomass productivity, low input requirements, short growth period, and tolerance to water deficits and various environmental stresses (Xu et al. 2018), but also because it is one of the most economical crops for producing high-quality bioethanol (Liu et al. 2015).

Sorghum can be categorized into three groups based on its use as food, feed, and energy (Shakoor et al. 2014). Sweet sorghum, a type of energy sorghum, is notable for its high soluble sugar content, which can be efficiently converted into bioethanol (Appiah-Nkansah et al. 2019). The sugar concentration in sorghum is determined by the Brix value, which reflects the percentage of soluble sugar (Mathur et al. 2017). However, developing sweet sorghum for bioenergy production faces challenges in meeting the standards of the alcohol and sugar industries. These standards include high stem weight capacity, resistance to lodging, high juice volume, high total soluble solids content in the stem, resistance to pests and diseases, and tolerance to drought (da Silva et al. 2017). These qualities are crucial for achieving sustainability and efficiency in bioenergy production.

Crossing and selecting superior sorghum genotypes is used in sorghum breeding to enhance biofuel traits, such as biomass, total soluble sugars, and stalk juice production (Bandara et al. 2020; Abu-Ellail et al. 2023). Breeders can achieve high genetic variability through these crosses and create gene combinations with desirable plant characteristics. Wide genetic variability in the progeny of a cross can lead to the development of superior genotypes through selection. Additionally, the transgressive index can predict transgressive segregants, individuals in the F2 sorghum population with better phenotypic values than their best parent. Recent studies have reported the identification of transgressive segregants for grain yield components in F2 sorghum populations, such as Maryono et al. (2019), Birhan et al. (2020), Munarti et al. (2022), and Rini et al. (2022). Some studies have also reported that selecting transgressive segregants can improve the adaptability of selected genotypes in rice (Koide et al. 2019), wheat (Putri et al. 2020), and corn (Sun et al. 2024). However, the identification of transgressive segregants for biomass-related traits for bioenergy, such as high biomass yield potential and efficient juice production, has yet to be reported. Discovering and selecting transgressive segregants could significantly enhance the efficiency and productivity of sorghum breeding for bioenergy.

Selection based on multiple characters is an effective method to obtain superior varieties with the desired plant ideotype, because it allows for the simultaneous improvement of several traits. Multi-trait selection using FAI-BLUP on sorghum yield has been reported by Botelho et al. (2021) for bioenergy purposes and Figueiredo et al. (2024) for feed purposes. The Multi-trait Genotype-Ideotype Distance Index (MGIDI) is a valuable selection method for producing superior genotypes. MGIDI is particularly useful in sorghum breeding for bioenergy production, enabling breeders to select multiple desirable traits simultaneously, thereby accelerating the breeding process (Olivoto and Nardino 2021). This study aimed to evaluate the genetic variability and multi-trait selection of biomass and stalk juice in a sorghum segregating population from the Samurai 2×Bioguma 1 Agritan Cross. The significance of this genetic variability and breeding efforts for sweet sorghum bioenergy production should inform researchers about the advancements in this field.

MATERIALS AND METHODS

Research sites

This research was performed from June to October 2023 at the Experimental Farm of the Center for Agricultural Instrument Standards, Biotechnology, and Genetic Resources, Ministry of Agriculture, Bogor, West Java, Indonesia. It is located at an elevation of 218 m above sea level. Post-harvest observations were performed at the Plant Breeding Laboratory, Institut Pertanian Bogor, Bogor, West Java, Indonesia. According to BMKG (2023), the research site experienced an average temperature of 34.3°C during the trials and an average rainfall of 200.8 mm. Soil conditions in the research field were analyzed in Testing Laboratory of Agronomy and Horticulture Department, Institut Pertanian Bogor and recorded as follows: pH H_2O (5.03), N-total (0.20%), P-Potential (131.18 mg P_2O_5/100 g), K-Potential (20.11 Mg K_2O/100 g), and Al-dd (0.22).

Research materials

The genetic material in this study consisted of 320 F2 genotypes resulting from a cross between Samurai 2 and Bioguma 1, conducted by the Department of Agronomy and Horticulture, Institut Pertanian Bogor. As check genotypes, three national varieties, namely Samurai 2, Bioguma 1 Agritan, and Numbu, as well as inbred sorghum lines from Institut Pertanian Bogor, namely IPB 1, IPB 4, and IPB 7 were used. These genotypes were chosen because they have high biomass potential, which is in accordance with the aims of this study.

Procedures

Experimental design

The study employed two experimental designs (i) experiments involving the F2 population; and (ii) experiments involving the check genotypes and parents. The F2 genotypes were planted in a population comprising 320 genotypes. The checks and parents were organized in a single-factor randomized complete block design, with the genotype factor consisting of 6 treatment levels and three replications.

The plot size used was 4×2.5 m, with planting performed at 70×20 cm spacing. Each hole was planted with 2-3 seeds. Carbofuran insecticide was applied at planting time, using a 0.5-1 g dose per hole. Plant maintenance activities included replanting, thinning, fertilizing, and managing pests, diseases, and weeds. Thinning was performed three weeks after planting, while weeding was performed periodically. Fertilizer was applied in two stages (i) 10 days after planting; and (ii) at 42 days after planting. Hilling was performed concurrently with the second fertilization application. Manual methods were used for weed control, while insecticides were applied as needed for pest control against grasshoppers, stinging grasshoppers, and aphids. Bird pest management involved protecting panicles with PE plastic covers. Harvesting occurred when 50% of the grains in each panicle reached full ripeness. The cultivation practices for sorghum adhered to the standards outlined by Wirnas et al. (2021).

Observation variables

The observation variables used in this study comprised quantitative traits, encompassing plant height (cm), stem diameter (mm), stay green duration (%), leaf greenness intensity (%), leaf area (cm^2), fresh biomass weight (g), stem weight (g), bagasse weight (g), juice volume (mL), Brix value, and grain weight per panicle (g). Each experimental unit was assessed with 20 sampled plants, while observations for the F2 population encompassed all plants.

Data analysis

The data analysis included seven main components as follows (i) Analysis of mean values using the t-student test at the 5% and 1% significance levels to compare quantitative traits between the F2 population and the two parental varieties. Minitab 21 software was used for this analysis; (ii) Estimation of gene action to evaluate the distribution of genotype frequencies in the F2 population. The normality of phenotype distributions for quantitative traits was assessed using skewness (S) and kurtosis (K) values, with statistical tests performed using the Z criteria at the 5% and 1% significance levels; (iii) Estimation of genetic parameters, including phenotype variance (σ^2p), environmental variance (σ^2e), genetic variance (σ^2g), broad-sense heritability (h^2bs), genetic coefficient of variability (GCV), and phenotype coefficient of variability (PCV). Where $(\sigma^2 p)=(\sigma^2_{\mathrm{F2}})$; $(\sigma^2 e)=(\sigma^2_{P1}+\sigma^2_{P2})/2$; $(\sigma^2 g)=(\sigma^2 p)-(\sigma^2 e)$; $(h^2_{\mathrm{bs}})=(\sigma^2 g/\sigma^2 p)*100\%$; GCV=(sqroot $\sigma^2 g/\bar{x}$)*100%; PCV=(sqroot $\sigma^2 p/\bar{x}$)*100%, with σ^2_{P1}: first parent variance, σ^2_{P2}: second parent variance, $\bar{x}$: general mean. The broad-sense heritability is categorized as high if >50%, medium if 20%-50%, and low if <20% (Stansfield 1991). The coefficients of genetic variability and phenotypic variability were categorized according to Knight (1979), where narrow (0%-10%), medium (10%-20%), and broad (>20%); (iv) Pearson correlation and path analyses were performed to examine the relationships between traits and assess direct and indirect effects on target traits. The analysis was performed using R software with the Agricole and Metan packages; (v) The transgressive index (TI) and number of segregants were estimated following the method described by Koide et al. (2019): $TI=(F_{2\ max}-F_{2\ min})/(P1-P2)$, with F_{2max}: highest value of F2 population, and $F_{2\ min}$: lowest value of the F2 population; P_1-P_2: the difference value between the two parents. Transgressive segregants were determined based on the number of individual plants with phenotypic values higher than the best and lower than the lowest; (vi) Multi-character selection based on the MGIDI involved analyzing all traits to evaluate the strengths and weaknesses of selected genotypes, selection differential, genetic gain, and selection progress. The analysis was performed using R software with the metan package. The selection differential (SD) was calculated using the following formula: $SD = \bar{x}_I - \bar{x}_0$, where $\bar{x}_I$ =the mean value of the selected population; $\bar{x}_0$=the mean value of the initial population; (vii) Cluster analysis was used to group genotypes based on genetic similarity between selected genotypes and check genotypes. Principle component analysis (PCA) was used to illustrate the distribution of genotypes based on character similarity. Heatmap visualization using the R package "pheatmap and factoextra".

RESULTS AND DISCUSSION

Analysis of mean values and variability of quantitative characters

The mean value, standard deviation, coefficient of variation, and distribution range of biomass traits in the F2 population and the parental lines are presented in Table 1. Results from the t-test, performed at a 95% confidence level, indicate significant differences between the two parents in various plant characteristics, including plant height, stay green, intensity of green leaf, stem weight, stalk juice volume, and grain weight per panicle. Bioguma 1 Agritan exhibited higher values than Samurai 2 in plant height, stem weight, stalk juice volume, and grain weight per panicle, whereas Samurai 2 showed superiority in other traits. The observed difference is attributed to the distinct genetic origins of the two parents. Bioguma 1 Agritan is an improved variety derived from gamma-ray irradiation of the Numbu variety, whereas Samurai 2 is a mutant of the Zh-30 lines. The t-test results further indicated significant differences in biomass traits, such as plant height, greenness intensity, leaf area, Brix value, and grain weight per panicle. In contrast, juice volume did not exhibit significant variation. Traits that did not show significant differences displayed similarities with the parental lines, whereas traits with significant differences reflected a combination of both parents. The coefficient of variation for agronomic and biomass traits ranged from 12.78% to 68.03%, indicating moderate to high variation among biomass traits in the F2 population (Ziegler and Tambarussi 2022).

Significant differences were also reported by Rini et al. (2022), which showed a variation in the mean value between the F2 population and parents. The genetic variation that occurs indicates that the F2 population has inherited different traits from its parents. The significant t-student test results indicate that there is a genetic contribution from both parents to their offspring. This difference may be caused by specific genes or gene combinations absent in the F2 population. The genetic variability highlighted by significant differences between the parents suggests the potential for developing new gene pools and creating novel genotypes in plant breeding programs (Yahaya et al. 2023). Furthermore, the comprehensive information observed in previous studies could be crucial for understanding the inheritance patterns and genetic potential of the F2 population, which can aid in enhancing sorghum yield components.

Table 1. Mean value, standard deviation, coefficient of variance, and range of quantitative characters in the F2 and parental populations

Characters	Mean and standard deviation				F2 coef var (%)	F2 range
	Samurai 2	Bioguma 1 Agritan	t-test	F2 populations		
PH (cm)	154.12±1.39	194.36±2.37	**	143.28^{ab}±18.31	12.78	90.00-237.00
SD (mm)	15.71±1.48	15.40±2.76	ns	15.66^{ns}±3.52	22.50	7.90-25.00
Stay green (%)	74.20±17.40	66.60±11.60	**	68.00^{a}±11.90	20.35	38.46-100
IGL (%)	43.92±10.50	37.62±10.30	**	40.72^{ab}±8.48	20.83	22.80-73.00
LA (cm^2)	478.50±82.10	470.00±111.00	ns	400.50^{ab}±96.40	24.06	193.45-620.50
FBW (g)	295.00±114.00	308.00±126.00	ns	274.00±111.00	40.64	90.00-570.00
SW (g)	154.30±61.00	197.00±83.50	**	142.70^{b}±68.20	47.81	50.00-360.00
SJV (mL)	42.30±33.40	63.20±46.90	**	41.92^{b}±28.50	68.03	17.00-295.00
BV (%)	14.91±2.39	15.43±2.07	ns	13.15^{ab}±2.43	18.49	6.00-18.00
DW (g)	72.70±29.80	83.30±22.70	ns	61.10^{b}±26.30	43.11	13.80-167.60
BW (g)	79.10±33.10	87.80±2.70	ns	81.30±31.90	39.21	10.00-190.00
GWPP (g)	33.00±10.90	41.70±15.50	**	29.00^{ab}±14.70	50.81	10.00-82.30

Notes: PH: plant height, SD: stem diameter, IGL: intensity of green leaves, LA: leaf area, FBW: fresh biomass weight, SW: stem weight, SJV: stalk juice volume, BV: brix value, DW: dry weight, BW: bagasse weight, GWPP: grain weight per panicle, *: Significantly different based on the t-test at α: 0.05, [a]: Significantly different from Samurai 2, [b]: Significantly different from Bioguma 1

Frequency distribution analysis of the mean values of biomass and agronomic characters revealed that plant height, fresh biomass weight, stem weight, juice volume, Brix value, dry weight, and grain weight per panicle exhibited lower values compared to their respective parents (Figures 1.A, 1.D, 1.E, 1.F, 1.G, and 1.H). In contrast, stem diameter and leaf greenness intensity (Figures 1.B and 1.C), stay green duration, and bagasse weight were in the range observed between the parental lines. Although some of the mean values for biomass traits in the F2 population were lower than those of their parents, the range of values indicated the presence of several genotypes with mean values surpassing those of their parents, indicating transgressive segregants. This result suggests that in the subsequent generation, these selected transgressive segregants may exhibit higher phenotypic values than their superior parents. This is due to the influence of epistasis, additive genes, or the expression of newly emerging recessive alleles in the F2 population (Cazzola et al. 2020; Rini et al. 2022). The identification and selection of transgressive segregation provides opportunities for plant breeders to develop new genotypes with higher biomass productivity.

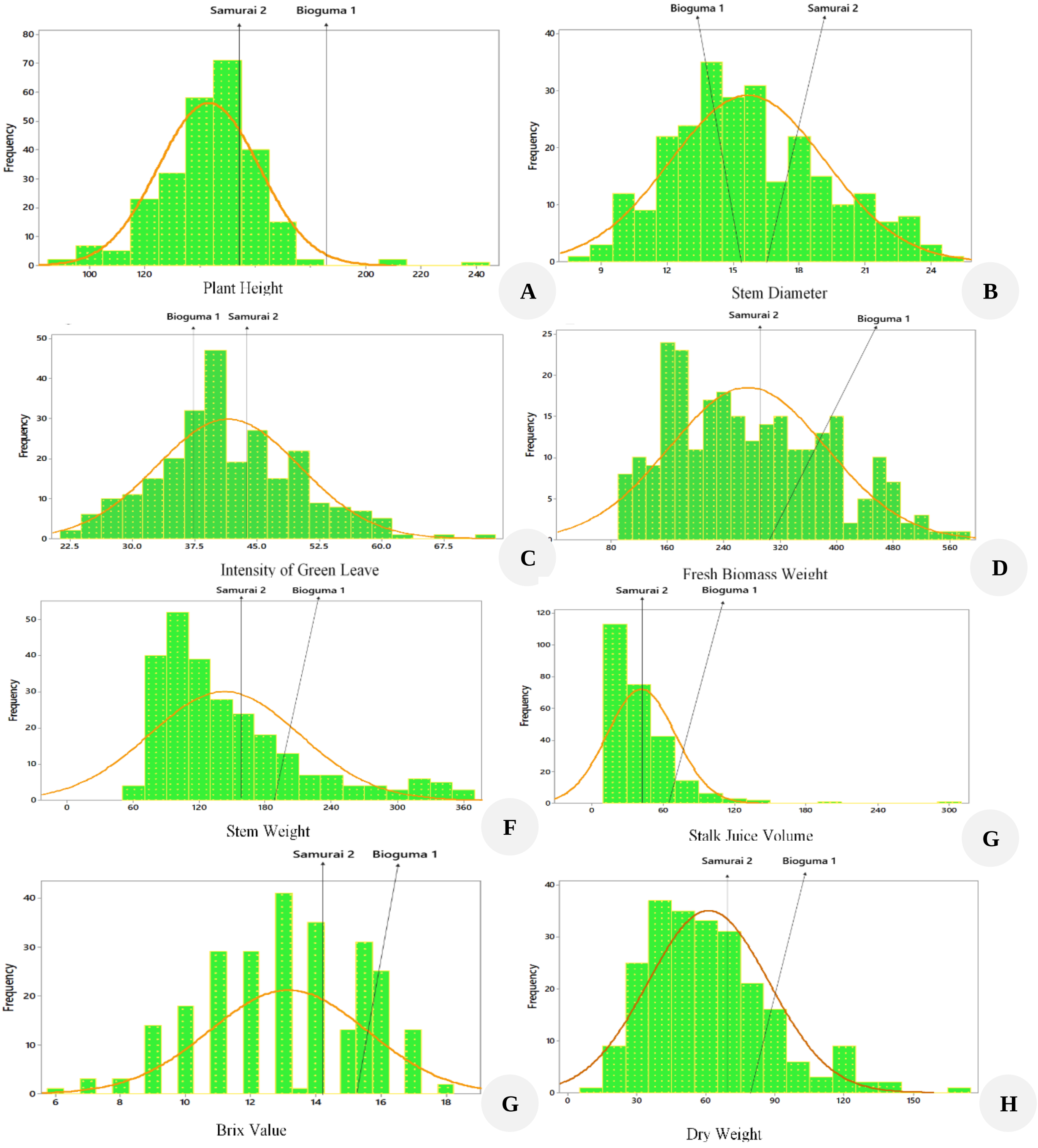


Figure 1. Distribution of quantitative data in F2 sorghum populations: A. Plant height; B. Stem diameter; C. Intensity of green leaves; D. Fresh biomass weight; E. Stem weight; F. Stalk juice volume; G. Brix value; H. Dry weight

Estimation of gene action in the F2 sorghum population

Analysis of gene action estimation using the skewness and kurtosis value approach is presented in Table 2. Skewness, which measures the asymmetry of the distribution, and kurtosis, which indicates the peak or flatness of the distribution, provide crucial insights into the factors influencing phenotype values. A skewed distribution, whether left or right, suggests the influence of environmental factors, gene-environment interactions (G×E), gene linkage, and epistasis. Positive skewness values indicate control by additive genes and complementary epistasis, while negative values suggest control by additive genes and duplicate epistasis. This analysis also helps predict the genetic mechanisms governing each trait (Jambormias 2015).

The Z critical value for plant height and leaf area was not statistically significant. The non-significant skewness value indicates that these traits exhibited a normal distribution and were primarily controlled by additive gene action (Herawati et al. 2019). The influence of additive genes played a crucial role in the genetic regulation of traits in sorghum breeding populations, contributing to their stability (Oliveira et al. 2019; Ishimori et al. 2020). Mohammed et al. (2015) also noted that additive and dominant gene effects play crucial roles in most sorghum traits. Selection for desirable characteristics in sorghum can effectively rely on the presence of additive genes. Traits influenced by additive genes can be inherited by progeny, allowing selection to occur in early generations.

Stem diameter and other traits, such as stay green duration, leaf greenness intensity, fresh biomass weight, stem weight, juice volume, dry weight, bagasse weight, and grain weight per panicle exhibited significant positive skewness (Table 2). Positive skewness values indicate complementary epistasis gene action, a crucial factor influencing phenotypic expression in sorghum populations (Chudasama et al. 2022). Understanding and selecting these traits can greatly enhance sorghum breeding programs. Jayaramachandran et al. (2010) noted that traits controlled by complementary epistasis gene action may undergo slower selection under low selection pressure. If selection is performed intensively, progress can accelerate. This finding underscores the importance of selection for traits controlled by complementary epistasis gene action to achieve significant genetic advancements. Insignificant skewness values indicate that the trait is primarily influenced by additive gene action (Herawati et al. 2019). Additive gene action suggests that the trait will be inherited, facilitating selection in early generations.

The study used kurtosis analysis to determine the number of genes influencing each trait. Results indicated that kurtosis did not differ significantly for stem diameter, stay green duration, leaf greenness intensity, Brix value, bagasse weight, and grain weight per panicle (Table 2). Specifically, stem diameter, stay green duration, and leaf greenness intensity were found to be influenced by multiple genes. The lack of significant kurtosis differences suggests a normal or mesokurtic distribution. A normal or mesokurtic distribution implies that these traits are governed by multiple genes (Mustafa et al. 2019). The number of genes controlling a trait has implications for the complexity of selection (Herawati et al. 2019). If a trait is polygenic, the influence of the environment will be significant, resulting in a lower probability of heritability. Conversely, if a trait is controlled by one or two genes (monogenic), the environmental influence is minimal, thereby leading to a higher heritability value.

The kurtosis values in the study offer valuable insights into the genetic control of traits. A positive kurtosis value, indicating a leptokurtic curve, suggests that a few genes regulate a trait. Conversely, a negative kurtosis value, indicating a platykurtic curve, suggests that many genes influence a trait. Hernandez (2023) observed that plant height, leaf area, fresh biomass weight, and Brix value were controlled by multiple genes, whereas fewer genes controlled other traits. This finding indicates that traits governed by polygenic genes and affected by additive gene action are more conducive to selection than those controlled by a few genes. The substantial genotypic variation in traits regulated by multiple genes enhances the effectiveness of selection, particularly when these traits exhibit high genetic variability. This underscores the significance of these findings for breeding programs.

Table 2. Estimation of gene action in the F2 sorghum population

Characters	S	Zs	Gene action	K	Zk	Number of controlling genes
Plant height	-0.16	-1.174^{ns}	Ad	2.28	8.3898**	A few genes
Stem diameter	0.32	2.348*	EK	-0.42	-1.545^{ns}	Many genes
Stay green	0.67	4.916**	EK	0.21	0.773^{ns}	A few genes
Intensity of green leaves	0.38	2.506*	EK	0.29	0.96^{ns}	A few genes
Leaf area	0.26	1.715ns	Ad	-0.8	-2.648**	Many genes
Fresh biomass weight	0.45	2.968**	EK	-0.62	-2.052*	Many genes
Stem weight	1.36	8.952**	EK	1.29	4.262**	A few genes
Stalk juice volume	4.19	27.582**	EK	28.02	92.572**	A few genes
Brix value	-0.36	-2.374*	Ad	-0.49	-1.622^{ns}	Many genes
Dry weight	0.92	5.745**	EK	0.97	3.041**	A few genes
Bagasse weight	0.5	3.202**	EK	0.19	0.611^{ns}	A few genes
Grain weight per panicle	0.91	6.002**	EK	0.3	0.993^{ns}	A few genes

Notes: S: Skewness; Zs: Statistic test for skewness; K: Kurtosis; Zk: Statistic test for kurtosis; **: Significant at 1% level; *: Significantly at 5% level; ns: Not significantly for skewness and kurtosis test; Ad: Additive, EK: Complementary epistasis; ED: Dominant epistasis

Estimation of variance components and heritability

The values of variance components, heritability, genetic variability coefficient, and phenotypic variability coefficient are presented in Table 3. Heritability, a critical concept in plant breeding, measures the proportion of total variation in a trait attributable to genetic factors. A high heritability value indicates that genetic variability predominantly influences the trait, thereby making selection more effective. Based on the heritability values, traits such as plant height, stem diameter, stay green duration, leaf greenness intensity, leaf area, Brix value, and grain weight per panicle exhibited high heritability, meeting the criteria for broad-sense heritability. Meanwhile, characters with moderate heritability values were observed in fresh biomass and bagasse weights, while others exhibited low broad-sense heritability values. Insan et al. (2016), Gebregergs et al. (2020), and Rini et al. (2022) reported that grain weight per plant also demonstrates high broad-sense heritability.

A high heritability value indicates the critical role of genetic factors in determining phenotypic traits. It also indicates that genetic factors strongly influence subsequent selection processes. According to da Silva Leite et al. (2020), early generation selection, such as in F2 populations, can be effective given adequate genetic variability and heritability of relevant traits. Furthermore, traits such as plant height, biomass yield, and sugar content can achieve substantial genetic improvement through recurrent selection. The potential of early selection, a potent strategy in plant breeding, is underscored by high heritability values. This is because the environmental influence on the expression of plant traits is minimal, enabling effective selection in the early generations (Birhan et al. 2020).

The coefficient of genetic variability (CGV) in the F2 population shows criteria that range from narrow to broad. Leaf area, fresh biomass weight, bagasse weight, and grain weight per panicle demonstrated a coefficient with broad criteria. Medium criteria for CGV were observed in plant height, stem diameter, stay green, leaf greenness intensity, and Brix value (Table 3). Furthermore, stem weight, juice volume, and dry weight exhibited narrow CGV values. A heritability value of 0% indicates that environmental factors significantly influence phenotypic variability, implying that observed trait differences are primarily due to environmental conditions rather than genetic factors. A narrow CGV indicates a lack of variability among individuals in the population, making selection ineffective. Uniformity in genetic variability means selection based on these characteristics is less effective. Conversely, if genetic variability is broad, selection will be more successful in increasing the frequency of the desired gene.

Based on the coefficient of phenotypic variability (CPV), nine characters exhibited a broad CPV value, while three showed medium CPV values. The range of CPV in the F2 sorghum population ranged from 14.23% to 95.94%. Characters with CPV values higher than the CGV indicate that environmental factors play a significant role in the variability observed in these traits, allowing for deviations if selection is performed based on these traits (Karnwal and Singh 2009). Trait improvement in plant breeding programs should prioritize characters with high heritability values and a substantial CGV.

Correlations between characters and direct and indirect effects on target characters

The results of the correlation analysis on the F2 population were statistically significant (Figure 2). Except for the Brix value, all characters showed a positive and significant correlation with fresh biomass weight and juice volume. de Resende and Alves (2020) categorized correlation values based on significance, with strong correlations (r>66%), medium correlations (34%<r<66%), and low correlations (r<34%). Particularly noteworthy is that plant height, stem diameter, leaf area, stem weight, juice volume, dry weight, and grain weight per panicle exhibited correlation values exceeding 70%, indicating a strong relationship with fresh biomass weight and juice volume. Stem weight exhibited notably high correlation coefficients with juice volume (r=0.88) and fresh biomass weight (r=0.96). Additionally, dry weight demonstrated a significant relationship with both stem and fresh biomass weights. This strong and positive correlation between these traits emphasizes the importance of integrating other positively correlated characteristics into the selection criteria, a critical consideration often overlooked in breeding programs.

Table 3. Estimation of variance components, heritability, genotypic coefficient of variance, and phenotypic coefficient of variance

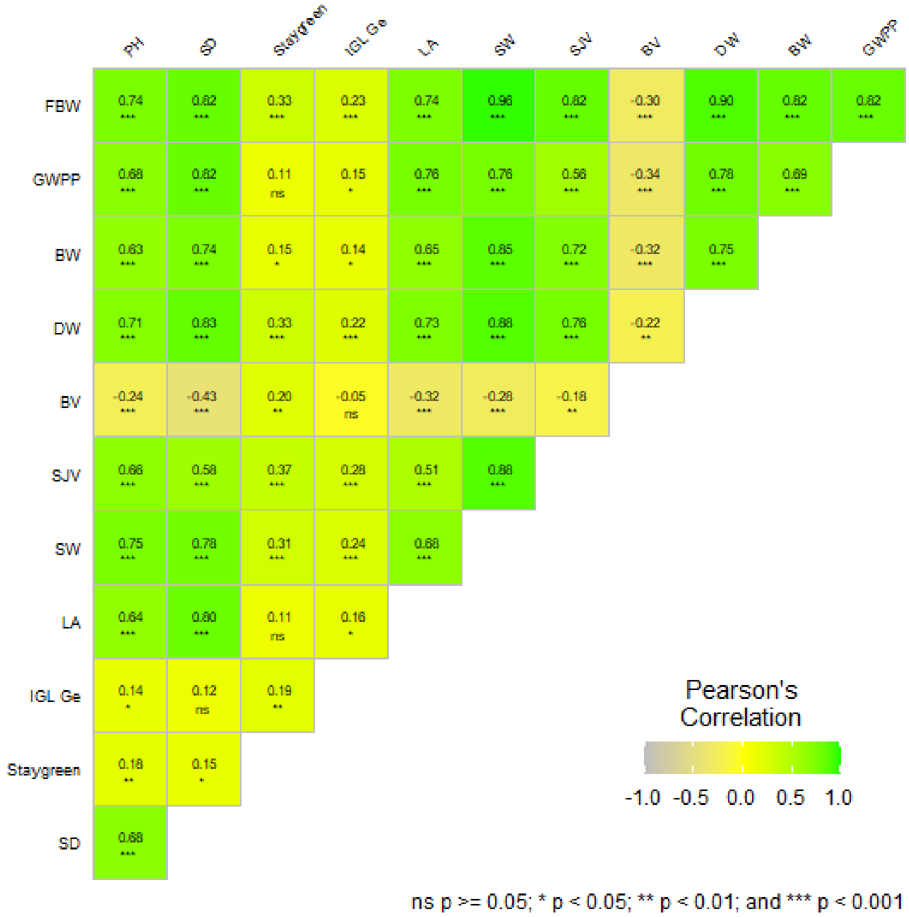
Characters	σ^2p	σ^2e	σ^2g	h^2_{bs}	CGV	CPV
Plant height	405.90	52.61	353.29	87.04^{H}	13.29^{M}	14.23^{M}
Stem diameter	12.95	4.89	8.06	62.23^{H}	18.39^{M}	23.28^{B}
Stay green	155.86	67.25	88.61	56.85^{H}	14.16^{M}	18.78^{M}
Intensity of green leaves	71.84	16.97	54.87	76.38^{H}	18.19^{M}	20.82^{B}
Leaf area	12026.00	5096.62	6929.38	57.62^{H}	21.93^{B}	28.89^{B}
Fresh biomass weight	5692.06	3327.39	2364.67	41.54^{M}	36.56^{B}	56.72^{B}
Stem weight	1671.21	2538.50	-867.29	0.00^{L}	0.00^{N}	61.72^{B}
Stalk juice volume	147.13	252.36	-105.23	0.00^{L}	0.00^{N}	95.94^{B}
Brix value	6.00	0.64	5.35	89.27^{H}	18.21^{M}	19.27^{M}
Dry weight	168.80	319.33	-150.53	0.00^{L}	0.00^{N}	44.01^{B}
Bagasse weight	470.52	260.06	210.46	44.73^{M}	33.57^{B}	50.19^{B}
Grain weight per panicle	232.04	58.61	173.44	74.74^{H}	47.05^{B}	54.42^{B}

Notes: σ2p: Genotypic variance, σ2e: Environmental variance, σ2p: Phenotypic variance, h2 bs: Broad sense heritability, CGV: Coefficient of genetic variances, CPV: Coefficient of phenotypic variances

The volume of juice in the stalk exhibited a negative correlation with the Brix value, indicating that as juice volume increases, the sugar concentration decreases owing to dispersion in a larger volume. Conversely, lower juice yields result in higher Brix values because the sugar is more concentrated. Plants with higher biomass yields typically produce more juice (López et al. 2021). However, if energy allocation prioritizes structural growth, the sugar concentration in the juice tends to be lower because it disperses in a larger volume (Lestari et al. 2021). Conversely, when biomass weight is low and metabolic processes prioritize sugar accumulation, it is possible to yield juice with a high Brix value. Thus, the negative correlation between Brix value and biomass and juice stalk yield, alongside the positive correlation with stay green, illustrates the trade-off or balance in energy allocation between sugar production and plant structural growth.

The yield of extracted juice plays a crucial role in achieving high bioethanol production (Carvalho and Rooney 2017). Conversely, the Brix value is equally important because bioethanol production involves fermenting sugar solutions. A higher Brix value in the juice results in greater ethanol yield. Guden et al. (2021) demonstrated a strong correlation between juice yield, fructose, and glucose concentrations, highlighting that increased juice yield and higher concentrations of fructose and glucose lead to greater bioethanol production. The Brix value typically correlates with the sucrose content in sweet sorghum stalks, which averages approximately 75%, along with minor amounts of fructose and glucose, approximately 2.6% (Kawahigashi et al. 2013). Guden et al. (2021) reported significant variability in juice yield across different environments and years, achieving the highest production in juice volume (22,740 L/ha), bioethanol yield (1,569.6 L/ha), and Brix value (15.63°Bx). This variability in juice yield was influenced by both genotype and environmental factors (Reddy et al. 2014).

Fresh biomass weight strongly correlated with juice volume (r=0.82) (Figure 2). de Oliveira et al. (2021) also reported similar findings, highlighting a strong correlation between green biomass production and juice volume. Dry weight exhibited a positive and significant correlation with juice volume (r=0.76), indicating that improvements in biomass weight traits may lead to corresponding enhancements in juice volume characteristics. Grain weight per panicle displayed positive and significant correlations with all characters except the Brix value. Similarly, Sulistyowati et al. (2016) observed a strong correlation between grain weight per plant and other yield components, suggesting its utility in estimating grain weight per panicle.



	PH	SD	Staygreen	IGL Ge	LA	SW	SJV	BV	DW	BW	GWPP
FBW	0.74 ***	0.82 ***	0.33 ***	0.23 ***	0.74 ***	0.96 ***	0.82 ***	-0.30 ***	0.90 ***	0.82 ***	0.82 ***
GWPP	0.68 ***	0.82 ***	0.11 ns	0.15 *	0.76 ***	0.76 ***	0.56 ***	-0.34 ***	0.78 ***	0.69 ***	
BW	0.63 ***	0.74 ***	0.15 *	0.14 *	0.65 ***	0.85 ***	0.72 ***	-0.32 ***	0.75 ***		
DW	0.71 ***	0.83 ***	0.33 ***	0.22 ***	0.73 ***	0.88 ***	0.76 ***	-0.22 **			
BV	-0.24 ***	-0.43 ***	0.20 **	-0.05 ns	-0.32 ***	-0.28 ***	-0.18 **				
SJV	0.66 ***	0.58 ***	0.37 ***	0.28 ***	0.51 ***	0.88 ***					
SW	0.75 ***	0.78 ***	0.31 ***	0.24 ***	0.68 ***						
LA	0.64 ***	0.80 ***	0.11 ns	0.16 *							
IGL Ge	0.14 *	0.12 ns	0.19 **								
Staygreen	0.18 **	0.15 *									
SD	0.68 ***										

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

Figure 2. Heatmap depicting correlations between agronomic traits and biomass in the F2 sorghum populations

The path analysis of the genetic correlation coefficient among biomass components through primary variables, such as juice volume and fresh biomass weight is presented in Figure 3. The coefficient of determination, which measures the goodness of fit of the regression line, indicates that the analyzed variables can explain 58.09% of juice volume and 78% of fresh biomass weight. The residual effects of 0.41 and 0.22 represent the additional contribution of model variables beyond the basic model. This model illustrates the causal relationships between essential variables, the target juice volume, and fresh biomass. Stem weight strongly correlated with juice volume (r=0.88) and exerted the highest direct effect (1.00). Thus, selecting genotypes with greater stem weight can lead to higher juice volume. This finding is consistent with de Oliveira et al. (2021), who reported that biomass production without panicles strongly influenced juice volume directly and indirectly.

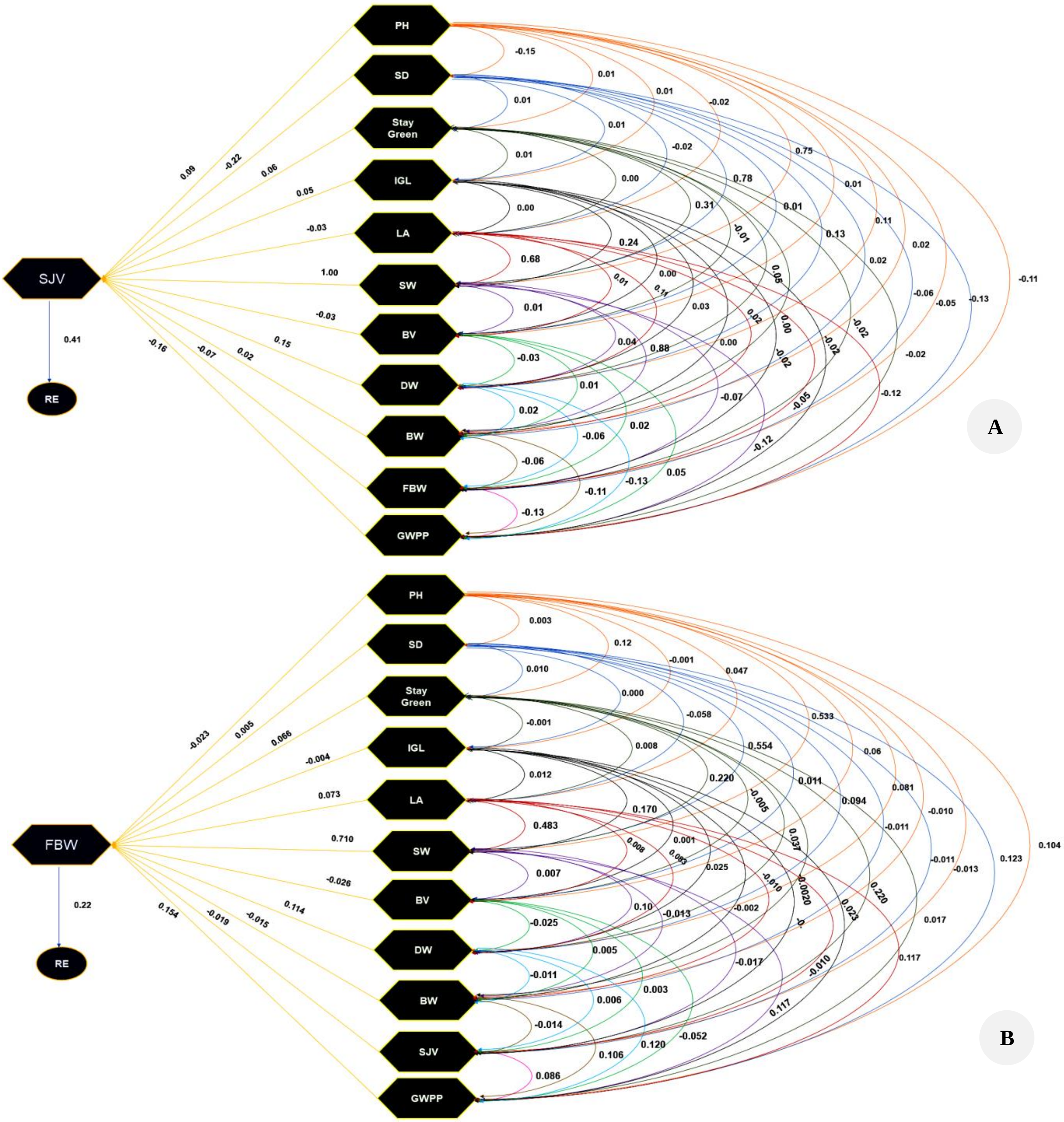


Figure 3. Direct and indirect effects on: A. Juice stalk yield; B. Fresh biomass yield with R-square values of 58.09% and 78%, respectively

Plant height, stem diameter, dry weight, and bagasse weight significantly correlated with juice volume, making them important in the indirect selection process. Despite the strong correlation, these traits exhibited a low and negative direct effect, suggesting that they were significantly influenced by the indirect effect of other traits, particularly stem weight. The stem weight trait had a high indirect effect on all variable pathways, leading to low or negative direct effect values for other variables. This indicates that the direct influence of these variables on the target trait is primarily mediated through stem weight. When the correlation coefficient is positive, but the direct effect is negative, the correlation is due to the indirect effect of the analyzed data (Lorentz et al. 2006). Characters with positive correlation coefficients but negative indirect effects contribute less to the base variable. Overall, the studied characters varied in their direct influence on juice volume. Stem weight had the strongest correlation and direct effect on juice volume, indicating a causal relationship in its production.

Identification of transgressive segregants for bioenergy sorghum

The transgressive segregants index on biomass and yield characters in the F2 population are presented in Table 4. The results showed that seven characters with a high transgressive index, four characters categorized as medium, and one with a low transgressive index. The classification of the transgressive index refers to Cazzola et al. (2020), which are high (TI>10), medium (5.0≤TI≤10.0), and low (0.0<TI<5.0). The transgressive index in the F2 population ranged from 3.65 to 55.16. Stem diameter exhibited the highest transgressive index (55.16), while plant height showed the lowest (3.65). Identification of transgressive segregations, which show phenotypic performance outside the range of the parents is an effective strategy for selecting superior genotypes for bioenergy in sorghum (Koide et al. 2019). Fresh biomass weight, juice volume, and brix value also have a high transgressive index. High and low transgressive index values depend on the magnitude of the difference in the mean value between the two parents (Munarti et al. 2022). Early selection against this segregation can improve plant breeding efficiency (Maryono et al. 2019; Birhan et al. 2020).

A significant transgressive index in the F2 population indicates the possibility of obtaining highly segregating individuals, which is essential for developing superior genotypes (Koide et al. 2019). The stem diameter and leaf greenness intensity characters exhibited a substantial number of segregants, exceeding the parental traits by 46.9% and 46.89%, respectively. In contrast, fresh biomass weight, juice volume, and Brix showed 5.43%, 1.94%, and 13.57% segregants, respectively. This diverse phenotypic expression in segregating populations likely stems from the accumulation of complementary alleles generating new genetic combinations and previously hidden harmful recessive alleles (Rick and Smith 1953). Genotypes displaying superior traits such as high fresh biomass weight, juice volume, and Brix can thus be selectively bred and developed into bioenergy sorghum in subsequent F3 and F4 generations.

Multi-trait selection of F2 populations for bioenergy sorghum

The selection of superior genotypes in plant breeding programs is a critical stage that involves identifying the best genotypes based on the breeder's specific target characters or criteria. Using selection indices and ideotype distances, breeders can identify potential genotypes with high selection index values, leading to the development of superior hybrids or inbred lines of sorghum. According to Botelho et al. (2021), multi-character selection is more accurate in estimating genetic parameters, predicting genetic values, and achieving higher selection values compared to selection based on individual characters. Furthermore, Figueiredo et al. (2024) reported that multi-character selection using FAI-BLUP on sorghum lines for feed purposes resulted in significant genetic gains. Therefore, employing multi-character selection methods is crucial for identifying superior genotypes for bioenergy sorghum purposes. One effective approach is the MGIDI index-based selection, which integrates ideotype and genotype distances across target characters (Olivoto and Nardino 2021). In this study, selection was performed with a 10% intensity, leading to the identification of the top 26 genotypes (Figure 4). Among the selected genotypes were G3, G194, G18, G40, G244, G80, G41, G27, G38, G83, G35, G98, G44, G5, G26, G21, G1, G64, G42, G47, G54, G43, G48, G30, G16, and G250.

Table 4. Transgressive segregants in biomass traits and yield of F2 sorghum populations

Characters	Range of F2 populations	Parents different	Mid parents	TI	T (%)
Plant height	147.00	40.24	174.24	3.65^{L}	1.94
Stem diameter	17.10	0.31	15.55	55.16^{H}	46.9
Stay green	61.54	7.60	70.40	8.10^{M}	39.15
Intensity of green leaves	50.20	6.30	40.77	7.97^{M}	46.89
Leaf area	427.05	8.50	474.25	50.24^{H}	24.03
Fresh biomass weight	480.00	34.00	301.50	14.12^{H}	5.43
Stem weight	310.00	42.70	175.50	7.26^{M}	5.43
Stalk juice volume	278.00	20.90	52.75	13.30^{H}	1.94
Brix value	12.00	0.52	15.17	23.08^{H}	13.57
Dry weight	153.80	10.60	78.00	14.51^{H}	0.38
Bagasse weight	180.00	8.70	83.45	20.69^{H}	5.04
Grain weight per panicle	72.30	8.70	37.35	8.31^{M}	25.58

Notes: TI: Transgressive segregant index; T(%): Percentage of transgressive segregant; H: High; M: Medium, L: Low

Selection differential values based on multi character selection are presented in Table 5. This study used a selection intensity of 10%, which represents a moderate level of selection pressure commonly applied in plant breeding. This intensity is expected to increase fresh biomass weight, juice volume, and Brix value in the next generation by 9.35%, 28.1%, and 22.2%, respectively. Initially, the F2 population comprised 320 genotypes. However, owing to constraints in observing biomass components, only 258 genotypes were evaluated, resulting in the selection of the top 26 genotypes at the 10% selection intensity. An increase in the median values of other characters also accompanied the increase in fresh biomass weight and juice volume. This aligns with the findings of correlation analysis, which demonstrated a positive correlation between all traits and the weights of fresh biomass and juice volume. Additionally, traits such as plant height, stem weight, dry weight, leaf greenness intensity, and stay green also increased. Consequently, higher fresh biomass weight and juice volume are expected in the next generation.

The cluster analysis and principal component analysis (PCA) results are presented in Figure 5. The results of the cluster analysis illustrated that there was high diversity among the selected F2 sorghum genotypes based on MGIDI simultaneous selection (26 genotypes), the parents, and four check genotypes. The dendrogram showed that the selected genotypes formed two main clusters (Figure 5.A). The first cluster consists of G18, IPB 4, G194, Numbu, Bioguma 1, and IPB 1. The second cluster is divided into two sub-clusters. The first sub-cluster consisted of 11 F2 genotypes, IPB7, and Samurai 2, while the second sub-cluster consisted of 13 F2 genotypes. The genotypes are grouped based on genotypes that have biomass characters with high mean values. Genotypes in the same cluster tend to have similar characteristics and are more closely related than genotypes in different clusters (Luquet et al. 2019).

Table 5. Selection differential and estimation of genetic gain

Characters	Factor	X_0	X_s	SD	SDperc	H_{2bs}	G	F3 Estimation
PH	FA1	143	146	2.71	1.89	0.87	2.36	145.36
SD	FA1	15.7	14.7	-0.973	-6.22	0.62	-0.61	15.09
LA	FA1	389	356	-32.5	-8.36	0.58	-18.73	370.27
SW	FA1	68.5	75	6.51	9.51	0.00	0.00	68.50
SJV	FA1	13.2	16.9	3.71	28.1	0.00	0.00	13.20
DW	FA1	30.5	34.1	3.59	11.8	0.00	0.00	30.50
BW	FA1	44.1	41.5	-2.64	-5.97	0.48	-1.26	42.84
GWPP	FA1	28.3	24.7	-3.54	-12.5	0.75	-2.65	25.65
FBW	FA1	141	154	13.2	9.35	0.42	5.48	146.48
Stay green	FA2	4.79	6.15	1.36	28.5	0.57	0.77	5.56
IGL.Ge	FA2	41.5	45.7	4.24	10.2	0.76	3.24	44.74
BV	FA2	12.7	15.5	2.82	22.2	0.89	2.52	15.22

Note: X0: Mean value before selection; Xs: Mean value after selection; SD: Selection differential; SDperc: Percentage of selection differential; FAI: Factor Analysis; G: Genetic gain

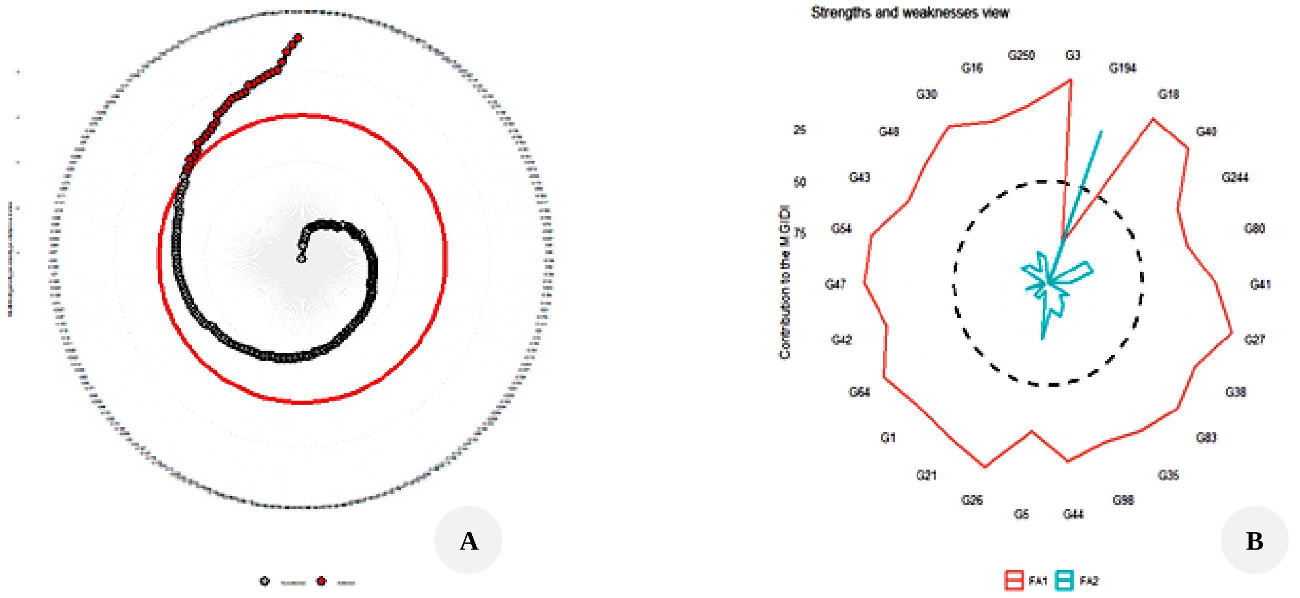


Figure 4. Selected sorghum genotypes based on A. MGIDI; B. Selected genotypes based on factor analysis. FA1 (PH, SD, LA, SW, SJV, DW, BW, GWPP, and FBW), FA2 (Stay Green, IGL, BV)

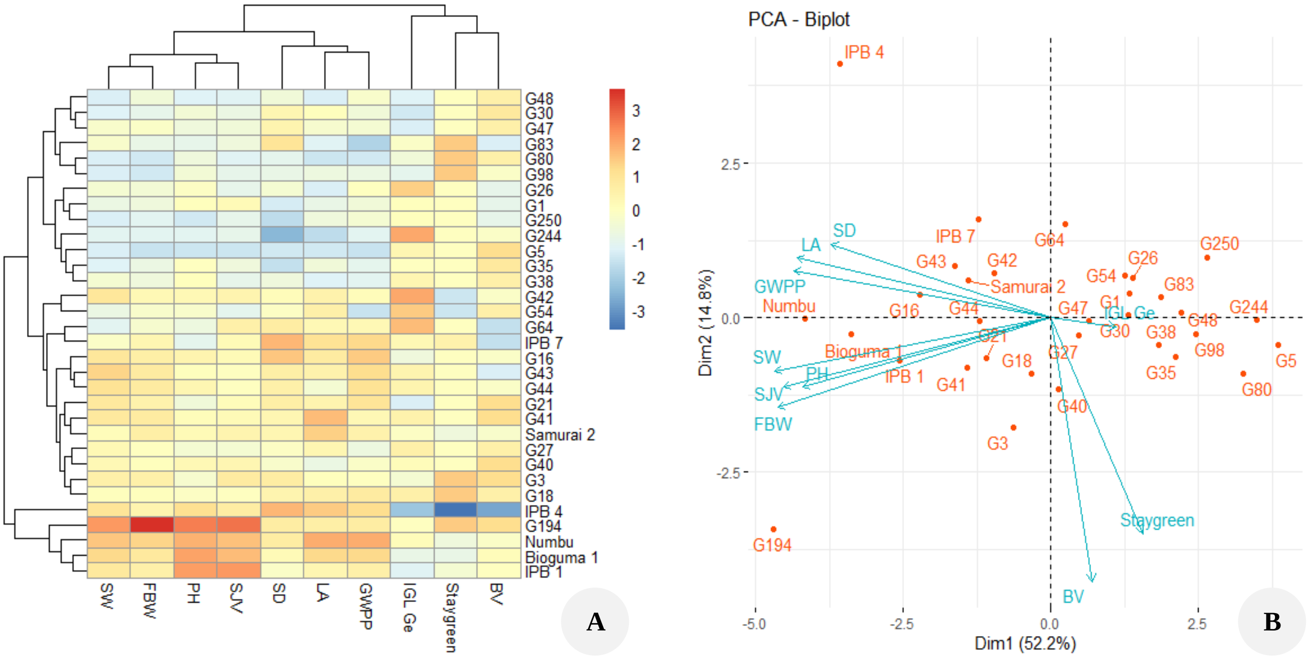


Figure 5. Cluster analysis A. Heatmap; B. Principal component analysis (PCA) Biplot, between F2 population and check genotypes

Genotypes 194 and G18 tend to have similarities with the national varieties Bioguma 1 Agritan and Numbu, as well as the inbred lines IPB 1, and IPB 4. This can be seen based on the color intensity in the dendrogram, where the intense red color tends to be evenly distributed in the first cluster, which indicates that the genotype is superior in biomass characters. G194 has a high juice volume and fresh biomass weight compared to the other genotypes. This indicates that these two genotypes have high biomass yield potential. Meanwhile, genotypes in the first sub-cluster with Samurai 2 and IPB 7 lines showed good biomass yields because the red color intensity was quite evenly distributed compared to the second sub-cluster, which had varied colors. This illustrates that genotypes in the second sub-cluster tend to have lower biomass yields. Selection based on cluster analysis is an effective method to identify superior genotypes based on similarity (Lestari et al. 2023). Through this grouping can be known kinship relationship between plant genotypes and can be used as a consideration in selection. Anshori et al. (2019) stated that selection would be effective if group characteristics could be known.

The clustering of sorghum genotypes was further tested by PCA biplot (Figure 5.B). The first and second principal components explained 52% and 14.8% of the variance, respectively. Genotypes with the same group in the cluster analysis were placed close together on the scatterplot. The results will validate the clustering based on the cluster analysis results. The axes of the biplot show the characters divided into three vectors. The first vector consists of FBW, SJV, and SW, the second vector consists of GWPP, LA, and SD, and the third vector consists of BV, staygreen, and IGL Ge. Thus, the axes in the first and second vectors indicate high biomass production and grain weight per panicle, while the third vector indicates high brix, staygreen and greenish intensity of the leaves. These results are in line with cluster analysis grouping, where cluster 1 grouping in the first and second vectors shows that most genotypes have high biomass and grain yield potential, but have low brix values. This occurs as a result of the tradeoff of the two traits (Lestari et al. 2024), where genotypes with high biomass yield and juice volume tend to have low brix values.

In conclusion, the F2 sorghum population exhibited high genetic variability in fresh biomass weight and juice volume, with coefficients of variation of 40.64% and 68.03%, respectively. All observed traits displayed broad-sense heritability values ranging from low to high (0-89.27%). Fresh biomass weight appeared to be influenced by multiple genes. In contrast, juice volume was controlled by a few genes exhibiting additive gene action and complementary epistasis, suggesting effective selection potential for these traits. Plant height, stem diameter, stay green, leaf greenness intensity, leaf area, Brix value, and grain weight per panicle demonstrated high heritability, establishing them as reliable selection criteria. Except for the Brix value, all characters showed significant positive correlations with fresh biomass weight and juice volume. Stem weight strongly influences juice volume and fresh biomass weight, allowing for the effective selection of these two traits through stem weight. Fresh biomass and juice volume traits have a high index, with transgressive segregants at 5.43% and 1.94%, respectively. Utilizing multi-trait selection based on the MGIDI index identified 26 top genotypes with selection differentials of 9.35% and 28.1% for fresh biomass weight and juice volume, respectively.

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