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Genetic Variability, Correlation, and Path Analysis in F_2 Population of Sorghum

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Abstract

Indonesian sorghum (*Sorghum bicolor* L.) germplasm with the highest genetic diversity can serve as a valuable resource for developing high-yielding cultivars. Therefore, the present research aimed to obtain information on genetic variability in the population and also determine the key traits associated with grain yield using correlation and path analysis in sorghum F_2 population. The sorghum parental genotypes 'Demak 4' and 'Soraya 3', and a local high-yielding IPB line, NS 19, NS 109, and NS 111, were used in the crosses. The F_2 population was evaluated against parental cultivars and four check genotypes. The results showed that 64 individual plants were observed as transgressive segregants. Meanwhile, the number of leaves and plant height were controlled polygenically through additive genes. The traits of panicle diameter, dry panicle weight, and grain weight per panicle possessed the highest genotypic coefficient of variance. The traits dry panicle weight and diameter were significantly positively correlated with grain yield. The considerable positive correlation with dried panicle weight, panicle length, and diameter could be used as selection criteria. The path analysis reported that the observed agronomic traits explained 97.07% of the total variation in grain weight per panicle.

Keywords: crossbreeding, food crops, local variety, sorghum rice, variance

Introduction

The world population is expected to reach 9.7 billion in 2050, with food demand projected to increase by 70% (Tripathi et al., 2019). In this context, Indonesia has a population of 278.11 million, projected to increase by 2.9 million annually (Statistics Indonesia, 2023). The population increase needs to be followed by a rise in crop production by increasing productivity and expanding land.

As a potential food source, sorghum (*Sorghum bicolor* (L.) Moench) is a subsistence crop cultivated by several African farmers. The crop ranks fifth globally as a food source, after wheat, rice, maize, and barley. The largest producers in the world are the United States, Nigeria, Sudan, and Mexico, with production at 12.1, 6.9, 6.4, and 5 million tons, respectively (Food and Agriculture Organization, 2022). In Indonesia, there has been no report on sorghum planting area, production, or productivity.

Sorghum is a grain crop with wide adaptability (Akinseye et al., 2023; Suguna et al., 2021) grown for generations by Indonesian farmers in Yogyakarta, West Nusa Tenggara, and East Nusa Tenggara Provinces (Fitrahtunnisa et al., 2020; Maftuchah et al., 2021; Martiwi et al., 2020). The crop must be addressed because policies focused on increasing rice production have led to high rice dependence. The food diversification program aims to reduce dependence by introducing alternative crops in line with consumer preferences. This

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includes researching and developing new crops, promoting nutritional benefits, and supporting cultivation and distribution.

The macronutrient and micronutrient content is comparable to that of rice and other cereals (Halim et al., 2022). This provides the highest content of protein, B vitamins, and iron (Fe) advantages than rice and corn (Taylor & Duodu, 2018). Additionally, sorghum is gluten-free, making the crop ideal for individuals with gluten allergies or celiac disease (Pezzali et al., 2020). The crop also has a low glycemic index (Amwoma et al., 2023) and contains antioxidant compounds (dos Reis Gallo et al., 2021). The development of the varieties to meet diverse consumer preferences, such as a slightly stickier rice texture, is crucial. Currently, no national variety has a texture determined by amylose content. Based on amylose content, sorghum is classified as waxy, heterowaxy, and non-waxy, with amylose contents of <2%, 10%-20%, and >20%, respectively (Rooney & Serna-Saldivar, 2000). Heterowaxy sorghum is expected to have a slightly stickier texture than rice. Previous research has shown that waxy traits are present in several local varieties (Suroya et al., 2023; Wirnas et al., 2024). Landraces can enhance rice texture but have low yield potential and poor pest and disease resistance. Hybridizing local and national varieties aims to increase yield and transfer desirable traits. Meanwhile, F_2 populations from these crosses are selected for high yield and improved seed quality, focusing on traits such as amylose content, Fe, and Zn.

Grain yield is a complex trait controlled by many genes whose expression is strongly influenced by the environmental factors (Acquaah, 2012). The selection progress mainly depends on genetic variability and the heritability of yield traits. In addition, high genetic advance and heritability indicate efficient selection in crop improvement (Mofokeng et al., 2019). Breeders need information on the traits of yield diversity and genetic control to ensure effective selection and meaningful progress. Selection can be carried out directly and indirectly on seed weight per plant and other characteristics, respectively. The goal is to improve yield potential and develop an ideal sorghum type. Wirnas et al. (2021) identified the ideal high-yielding sorghum as having a medium plant height (150-235 cm), stem diameter ≥18 mm, flag leaf area ≥200 cm², leaf greenness ≥45 CCI units, and panicle diameter ≥50 mm. The relationship between grain yield and yield components, as well as other morphological characteristics, should be considered when indirectly selecting ideal plants. Therefore, this research aimed to analyze the performance of sorghum F_2 segregating population for various agronomic traits resulting from crosses between low-amylose local sorghum and new superior varieties.

Material and Methods

Study Area

The present research was conducted from June to September 2023 at the Agricultural Instruments Standardization Agency of Biotechnology and Genetic Resources (BBPSI Biogen), Cimanggu, Bogor City, West Java Province, Indonesia. Subsequently, post-harvest measurements were conducted at the Laboratory of Plant Breeding, IPB University, Bogor, Indonesia.

Plant Materials

The genetic materials comprising the F2 population were obtained from the crosses between 'Demak 4' (local variety) x 'Soraya 3' (improved variety) and the checks. The checks grown included 'Demak 4', 'Soraya 3', IPB breeding lines (NS 19, NS 109, and NS 111), and a national sorghum variety ('Numbu').

Procedures

The seeds were sown with a row spacing of 75 cm and hill spacing of 15 cm with one seed per hill. The experiment on the F_2 population and parents used 15 plants of the check varieties from each replication, as well as 200 F_2 plants of 'Demak 4' x 'Soraya 3'. The check varieties were used as controls to calculate error values

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in F_2 "individual plants" of genetic experiments with non-replicated under an augmented design based on an individual-based segregated design. The check varieties were arranged in a Randomized Complete Block Design, with genotypes as blocks and three replications.

Crop maintenance involves fertilizing and controlling pests, diseases, and weeds. The fertilizer consists of 200 kg/ha urea (45% N), 100 kg/ha SP-36 (36% P_2O_5), and 100 kg/ha KCL (60% K_2O). Urea was applied in two stages: one-third at planting and two-thirds 3-4 weeks after planting or simultaneously with hilling. Pest and disease control were based on field conditions. Bird pest control was conducted by enclosing the panicles using PE plastic (Wirnas et al., 2021). Harvesting occurred when the seeds were fully matured, indicated by the emergence of a black layer on the lower part of the seed.

Observations were made on 200 F_2 plants, 10 samples per replicate for each parent, and the check varieties. The agronomic and yield-related traits were observed based on the Sorghum descriptor (UPOV 2015) guide number TG/122/4, which included the plant height, leaf number, leaf length, leaf width, stem diameter, panicle length, panicle diameter, dry panicle weight, 1000-grain weight, and seed weight per panicle.

Data Analysis

The data analysis consisted of comparing the mean values between parents and the F_2 population, which were tested using the *t*-test and visualized as a histogram in Minitab. Estimation of gene action in sorghum for agronomic traits and yield, estimation of variance components, heritability, and genotypic coefficient of variance (GCV), correlation analysis between traits, and path analysis of the target traits were performed using R software with the Agricolae package (de-Mendiburu, 2021). Mean adjustment was applied to account for potential biases that could distort analytical results.

Results and Discussion

Quantitative Traits in F_2 Sorghum Populations

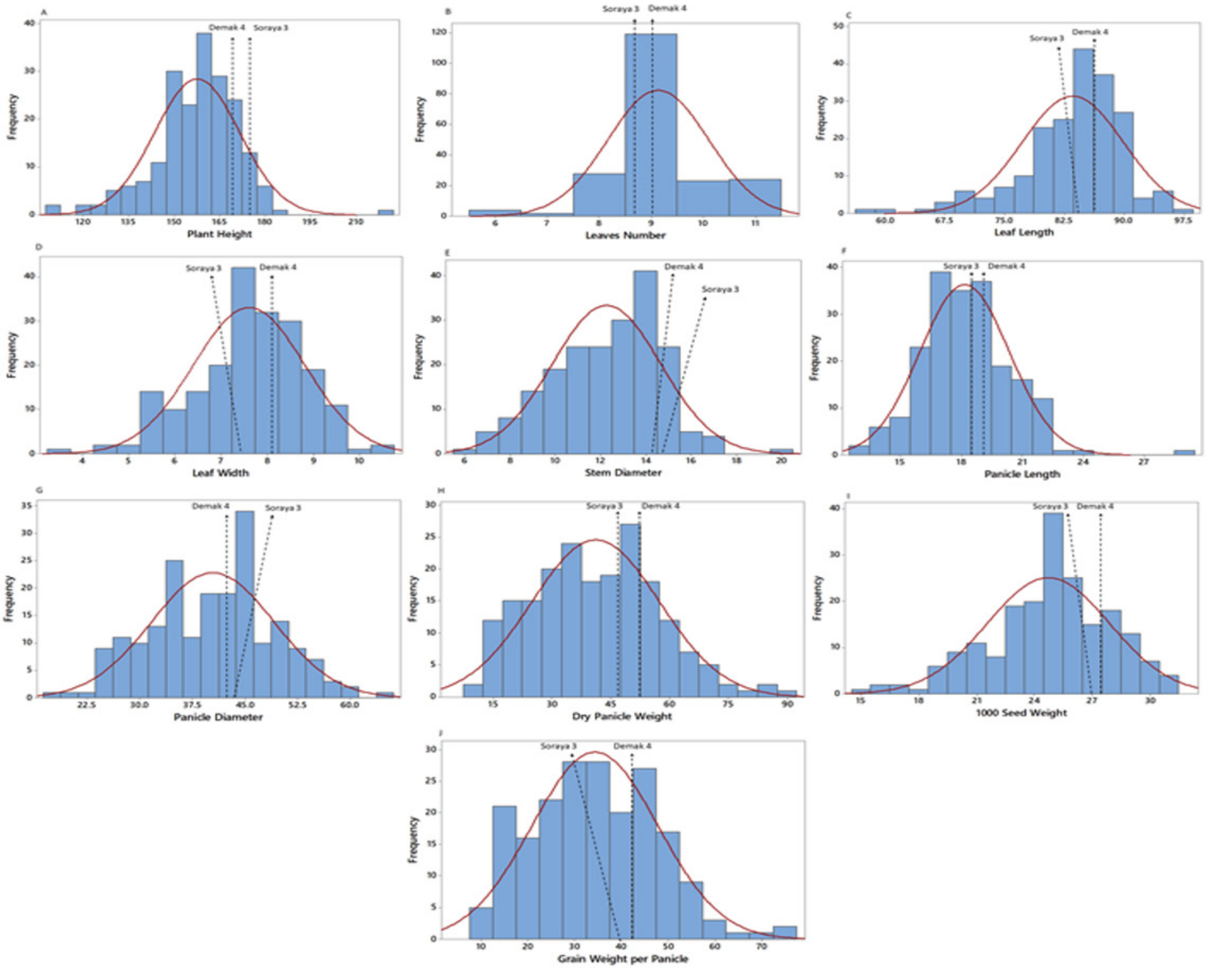
In sorghum F_2 populations, the quantitative traits had the highest variation in the scatter data compared to the parental genotypes. The mean values of plant height, stem diameter, panicle diameter, dry panicle weight, 1000-seed weight, and grain weight per panicle were lower than those of the parent cultivars. However, the leaf number, leaf length, leaf width, and panicle length were reported as average values in the F_2 population and were close to those of the parental genotypes. In the F_2 population, plant height was lower than in the two parents (Figure 1). Since the F_2 generation developed genotypes with medium plant stature ranging from 150 to 225 cm. This was because medium-sized plants in the Gramineae family typically produce large numbers of seeds, mature early, and are resistant to lodging. Jiang et al. (2024) reported that plant height was the most important trait in sorghum crop improvement.

Morphological traits such as leaf length, leaf width, and stem diameter exhibited the highest variability in the F_2 populationcompared to the parental genotypes. The wide ranges of F_2 in leaf length, leaf width, and stem diameter were 58-97 cm, 3.5-10.5 cm, and 5.6-19.9 mm, respectively. Each individual showed greater diversity in the F_2 population and exhibited the highest level of segregation. The same results were observed for panicle length and diameter, ranging from 13-29 cm and 18-64.9 cm, respectively. Some plants have better panicle traits than both parents. The male and female parents, 'Demak 4' and 'Soraya 3', have the panicle diameter of 43.61 mm and 43.62 mm, respectively.

In some yield components, the F_2 population have higher values than the parental genotypes. The wide range of F_2 "individual plants" for dry weight per panicle, 1000-seed weight, and grain weight per panicle was observed, with ranges of 10-90 g, 14.6-30.9 g, and 8-76 g, respectively, indicating the probability of a high-yielding transgressive segregant (Figure 1). Moreover, transgressive segregant selection was a breeding

Figure 1

Quantitative Data Distribution in The Sorghum F_2 Population



Notes. (A) plant height (cm), (B) leaves number, (C) leaf length (cm), (D) leaf width (cm), (E) stem diameter (mm), (F) panicle length (cm), (G) panicle diameter (mm), (H) dry panicle weight (g), (I) 1000 seed weight (g), (J) grain weight per panicle (g).

strategy for high-yielding varieties in self-pollinated crops such as sorghum. Information on parental genetic diversity in plant breeding programs using hybridization methods is crucial. Using parents with similar morphology in crosses produced many transgressive segregants in the early generations, thereby enhancing selection efficiency.

Grain weight per panicle is a yield character used to determine the plant productivity. Based on the grain weight per panicle in the F_2 'Demak 4' x 'Soraya 3' population, 64 plants were observed as transgressive segregants with grain weight per panicle of 42-76 g (Figure 1). Maryono et al. (2019) also reported that the grain weight per panicle, used as a yielding character, produced 134 transgressive segregants in the cross 'B69' x 'Kawali', with a grain weight per panicle of 66.6 g.

Gene Action in F_2 Sorghum Population

In this study, skewness was positively associated with panicle length but negatively associated with leaf length, leaf width, and 1000-seed weight (Table 1). Saikiran et al. (2023) stated that the traits in identifying population genetic potential with a breeding method for

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populations require knowledge of the nature and magnitude of gene action. Traits with nonsignificant skewness values also indicated that the data were normally distributed and were controlled by additive gene action. In this context, simple selection procedures and recombination breeding can be used as selection methods in the same population. Chaudari et al. (2018) reported that gene action indicated that the traits were highly heritable and that selection may be effective in early generations. Conversely, the traits are controlled by complementary epistasis when skewness is positively significant. Riyanto et al. (2023) explained that traits controlled by complementary gene action exhibited small genetic gains under low selection intensity. However, the traits leaf length, width, and 1000-grain weight showed harmful skewness, with negative values indicating duplicate epistasis. Duplicate epistasis is the interaction between two genes producing the same material to form the phenotype (Sayurandi & Woelan, 2016).

Dasriani et al. (2020) stated that gene action with the duplicate epistasis had the ability to mask each other. Duplicate epistasis only occurs when the genes produce the same material to form the phenotype. Similarly, complementary epistasis occurs when a gene's function is required for metabolism. Trikoesoemaningtyas et al. (2017) stated that traits controlled by epistasis gene action produced superior offspring compared to the parental genotypes. Therefore, trait selection managed by epistasis should be implemented in subsequent generations. Maryono et al. (2019) reported that epistasis was not inherited, and the selected traits could not be reproduced.

Kurtosis analysis was used to estimate the number of genes controlling a trait. The results showed that kurtosis did not vary significantly across leaf width, stem diameter, panicle diameter, dry panicle weight, 1000-seed weight, and grain weight per panicle. Normal or mesokurtic distribution was reported when the kurtosis value was not significantly different (Riyanto et al., 2023). Positive and negative kurtosis showed leptokurtic and platykurtic curves, respectively. Significant positive kurtosis was observed for plant height, number of leaves, leaf length, and panicle length (Table 1). The results showed the leptokurtic distribution of the various traits in F_2 population. According

Table 1

Skewness, Kurtosis, Gene Action, and The Number of Controlling Genes in The F_2 Sorghum Population

Traits	S	Zs		SEs	K	Zk		SEk	Gene action	Number of controlling genes
Plant height	-0.32	-1.86	ns	0.17	2.71	7.92	**	0.34	Ad	a few
Leaves number	-0.07	-0.41	ns	0.17	1.50	4.38	**	0.34	Ad	a few
Leaf length	-1.10	-6.40	**	0.17	1.96	5.73	**	0.34	ED	a few
Leaf width	-0.45	-2.62	**	0.17	0.21	0.61	ns	0.34	ED	many genes
Stem diameter	-0.28	-1.63	ns	0.17	-0.03	-0.09	ns	0.34	Ad	many genes
Panicle length	0.56	3.26	**	0.17	2.18	6.37	**	0.34	EK	a few
Panicle diameter	-0.02	-0.12	ns	0.17	-0.39	-1.14	ns	0.34	Ad	many genes
Dry panicle weight	0.31	1.80	ns	0.17	-0.23	-0.67	ns	0.34	Ad	many genes
1000 seed weight	-0.55	-3.20	**	0.17	0.21	0.61	ns	0.34	ED	many genes
Grain weight per panicle	0.28	1.63	ns	0.17	-0.21	-0.61	ns	0.34	Ad	many genes

Notes. S = skewness; Z_s = statistic test for skewness; K = kurtosis; Z_k = statistic test for kurtosis; SEs= standard error of skewness; SEk= standard Error of kurtosis; ** = significant at 1% level; * = significant at 5% level; ns = not significant for skewness and kurtosis test. Ad = additive, EK = complementary epistasis, ED = duplicate epistasis.

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to Samak et al. (2011), leptokurtic distribution reflects a trait controlled by a few genes.

Variance Components, Heritability, and Genotypic Coefficient of Variance (GCV)

All the traits exhibited high broad-sense heritability (Table 2). The F_2 populations produced the highest heritability for plant height (Trikoesoemaningtyas et al., 2017). A high heritability value suggests that genetic factors possess a greater impact on the phenotype than the environmental influences. This is crucial for effective selection because the environment has a minimal effect on the traits. Genetic variance indicated that selection was effective for the trait in the initial population (Imani et al., 2021). Traits with high broad-sense heritability values contributing positively to enhanced yield can be used as selection criteria to produce the ideal-type plants (Wirnas et al., 2021).

Heritability and genetic variability can enhance the selection-based improvement. This is because the variables are strongly influenced by the gene variation reflected in phenotypic diversity within an environment (Belay, 2018). The estimated value of broad heritability provides an overview of the inheritance of agronomic traits from parents to offspring. Traits with high heritability values indicated that genetic factors exert a more significant influence. Meanwhile, traits with medium heritability values indicate that the influence of genetic and ecological factors was similar on performance. In this context, crop improvement depends on genetic variability within populations. The coefficient of genetic variation and heritability estimates provide a better indication of the traits. Heritability can be determined more accurately when analyzed using genetic data, rather than relying on the average percentage (Saikiran et al., 2023).

This GCV ranged from 7.59% to 39.20%, and the values were classified as narrow, medium, and broad (Knight, 1979). The traits panicle diameter, dry panicle weight, and grain weight per panicle showed the highest GCV values among the traits. Plant height and leaf length have the lowest GCV values and are in the narrow category, while the other traits are in the medium category. The broad GCV value indicated that the observed agronomic traits exhibit high diversity, and their behavior tends to be diverse as well. This might have been due to the segregation found in the F_2 population. The traits with higher GCV values offer greater chances of successful selection (Maryono et al., 2019).

Table 2

The Estimated Variance Components, Broad Sense Heritability Values, and Genotypic Coefficient of Variance in the F_2 Sorghum Population

Traits	Mean	Std.Dev	σ^2_p	σ^2_e	σ^2_g	h^2_{bs}	Criteria	GCV (%)
Plant height (cm)	157.65	14.05	197.27	0.53	196.73	99.73	High	8.90
Leaves number	9.14	0.97	0.94	0.09	0.86	90.95	High	10.13
Leaf length (cm)	83.62	6.37	40.61	0.37	40.24	99.08	High	7.59
Leaf width (cm)	7.62	1.21	1.46	0.18	1.28	87.35	High	14.83
Stem diameter (mm)	12.27	2.40	5.74	0.44	5.30	92.33	High	18.76
Panicle length (cm)	18.18	2.20	4.83	0.26	4.57	94.67	High	11.77
Panicle diameter (mm)	40.45	8.75	76.54	0.33	76.21	99.56	High	21.58
Dry panicle weight (g)	41.22	16.24	263.86	0.47	263.40	99.82	High	39.37
1000 seed weight (g)	24.74	3.18	10.12	0.74	9.38	92.66	High	12.38
Grain weight per panicle (g)	34.39	13.49	181.93	0.19	181.74	99.90	High	39.20

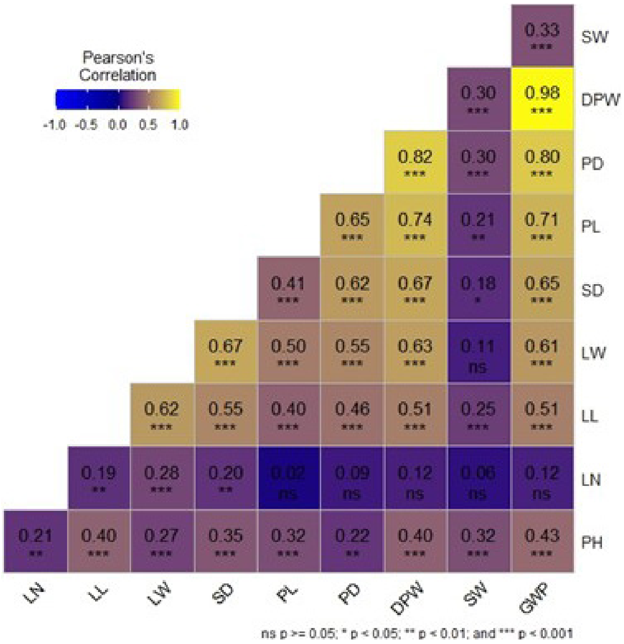
Correlation and Path Analysis Between Agronomic and Yield Traits

Correlation analysis provides an overview of the relationship among the agronomic traits. The relationship shows the connection between the supporting and the grain yield. The correlation coefficient shows the degree of the relationship between the traits. The correlation analysis can also be used to predict a particular character through others (Sulistyowati et al., 2016). This showed the relationship between the essential traits that facilitate the selection and improvement of complex polygenic traits, such as grain yield in sorghum. In addition, selection efficiency can be improved by understanding the extent of the relationship between the grain yield and its component traits. Correlation between the agronomic traits and high heritability enhances the efficiency of the selection process (Ngidi et al., 2024) a prerequisite to developing sorghum varieties with desirable agronomic traits and high carbon sequestration for sustainable crop production and soil health. The present study aimed to assess the extent of genetic variability and associations among agronomic and carbon storage traits in selected sorghum genotypes to identify the best candidates for production or breeding. Fifty genotypes were evaluated at Ukulinga, Bethlehem and Silverton sites in South Africa during the 2022/2023 growing season. The following agronomic and carbon storage traits were collected: days to 50% heading (DTH). Information on the correlations among agronomic traits is crucial for plant breeding programs.

The present research found a significant positive correlation between grain weight per panicle and all other traits except the number of leaves (Figure 2). The grain yield (grain weight per panicle) was found to be positively correlated with the plant height (r = 0.43), leaf length (r = 0.51), leaf width (r = 0.61), stem diameter (r = 0.65), panicle length (r = 0.71), panicle diameter (r = 0.80), dry panicle weight (r = 0.98), and 1000-seed weight (r = 0.33). The traits with the

Figure 2

Correlation in the F_2 Population



Notes. PH = plant height; LN = leaves number; LL = leaf length; LW = leaf width; SD = stem diameter; PL = panicle length; PD = panicle diameter; DPW = dry panicle weight; SW = 1000 seed weight; GWP = grain weight per panicle.

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highest correlation coefficients for grain weight per panicle were dry panicle weight (r = 0.98) and panicle diameter (r = 0.80). However, leaf length, leaf width, and stem diameter, as photosynthetic traits, showed significant correlations with grain weight per panicle. Genotypes with higher photosynthetic capacity tended to have greater grain weight per panicle.

Several traits were identified to be correlated with higher yield potential in sorghum. According to Wirnas et al. (2021) strong correlations between the traits suggested that panicle weight could be targeted to increase the yield potential. This was supported by the heatmap correlation analysis, which showed that grain weight per panicle was reflected. Enyew et al. (2021) reported a strong correlation between traits, with a coefficient of r = 0.99. Sulistyowati et al. (2016) and Suroya et al., 2023) recorded that panicle weight had a significant positive correlation with the grain weight per panicle (r = 0.99 and r = 0.97). Akatwijuka et al. (2019) reported that considerable photosynthetic machinery could be necessary for grain filling and enhanced crop production. In addition, there was a substantial correlation between the grain yield, leaf length, and width. A strong positive association was also reported in earlier sorghum research between the grain yield and various related traits.

The results showed that, in sorghum, dry panicle weight and panicle diameter were significantly correlated with grain yield. The character with a significant direct effect on grain weight per panicle was the dry panicle weight (1.000) (Table 3). This has a correlation coefficient value to the weight of grains per panicle, which was large and similar to the direct effect (0.98). Singh and Chaudhary (1979) explained that the correlation coefficient of agronomic traits described the closeness of the relationship. Similar results were obtained by Senbetay (2020), in which panicle weight positively affected grain yield. Therefore, traits such as panicle weight, number of grains, and length were important and can be considered in planning breeding strategies to increase grain yield.

The traits plant height, leaf length, leaf width, stem diameter, panicle length, panicle diameter, and 1000-seed weight were found to be positively correlated with grain weight per panicle and to have minimal, if any, direct effects. Singh and Chaudhary (1979) reported a positive correlation coefficient. Meanwhile, the correlation between the various traits and grain yield was caused by indirect effects. The value of indirect influence through dry panicle weight was excellent for panicle length and diameter (0.53 and 0.66, respectively). Therefore, these

Table 3

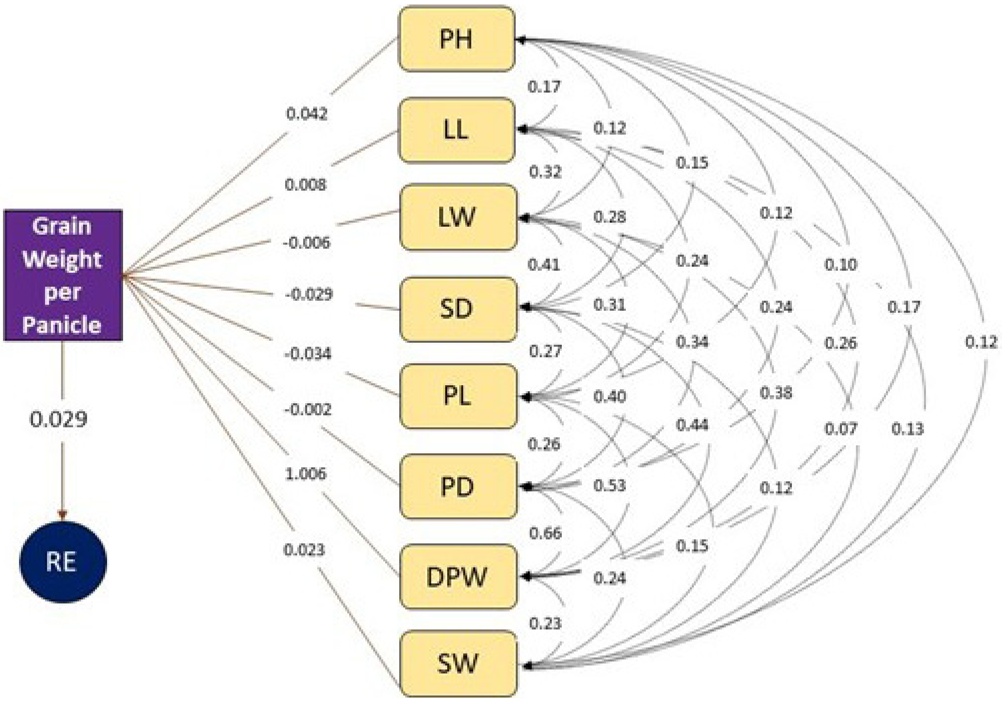
Direct and Indirect Effects of Different Segregation on Grain Yield in the F_2 Population

Traits	PH	LL	LW	SD	PL	PM	DPW	SW	Correlation with GWP
PH	**0.042**	0.172	0.116	0.151	0.138	0.095	0.172	0.138	0.430
LL	0.204	**0.008**	0.316	0.281	0.204	0.235	0.260	0.128	0.510
LW	0.165	0.378	**-0.006**	0.409	0.305	0.336	0.384	0.067	0.610
SD	0.228	0.358	0.436	**-0.029**	0.267	0.403	0.436	0.117	0.650
PL	0.227	0.284	0.355	0.291	**-0.034**	0.462	0.525	0.149	0.710
PM	0.176	0.368	0.440	0.496	0.520	**-0.002**	0.656	0.240	0.800
DPW	0.392	0.500	0.617	0.657	0.725	0.804	**1.000**	0.294	0.980
SW	0.106	0.083	0.036	0.059	0.069	0.099	0.099	0.023	0.330

Notes. Residual values 0.029, Bold and diagonal values indicate direct effects, PH = plant height; LN = leaves number; LL = leaf length; LW = leaf width; SD = stem diameter; PL = panicle length; PD = panicle diameter; DPW = dry panicle weight; SW = 1000 seed weight; GWP = grain weight per panicle.

Figure 3

Path Diagram: Agronomic Traits of Grain Weight per Panicle



Notes. Agronomic traits that act as X are traits that have a significant correlation with the character of grain weight per panicle (Y), plant height (PH), leaf length (LL), leaf width (LW), stem diameter (SD), panicle length (PL), panicle diameter (DD), dry panicle weight (DPW) and 1000 seed weight (SE).

trait values were accompanied by an increased dry panicle weight.

The relationship between agronomic traits influencing grain weight per panicle (Figure 3). The path diagram showed a residual coefficient (RE) of 0.029 for the F_2 population. Therefore, the observed agronomic traits explained 97.07% of the variation in grain weight per panicle, with the remaining (2.93%) being influenced by unobserved factors.

Conclusions

In conclusion, 64 sorghum individual plants were observed as transgressive segregants, with the highest grain weight per panicle compared to the parental genotypes. The traits panicle diameter, dry panicle weight, and grain weight per panicle had the highest GCV values. A significant positive correlation of grain weight per panicle with all other traits was observed, except for the number of leaves. The character directly affecting the grain weight per panicle was dry panicle weight. However, the value of indirect influence through the character of dry panicle weight was excellent for panicle length and diameter. In this context, dry panicle weight was increased by both traits.

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