

Genetic Analysis in an F_2 Population Derived from Indonesian Local Sorghum with New Superior Variety

Analisis Genetik Populasi F_2 Hasil Persilangan Sorghum Lokal Indonesia dengan Varietas Unggul Baru

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ABSTRACT

Genetic analysis and inheritance patterns studies on agronomic and yield characters were required to develop a plant breeding program. This study aimed to obtain information on agronomic and yield performance, gene action, genetic diversity, and heritability in the F_2 population derived from crosses of local and high-yielding varieties. The study was conducted in August-November 2021 in Dramaga, Bogor. The F_2 sorghum population, female and male parents, and also ten check genotypes were used in this study. The results showed qualitative characters in the F_2 population indicated the female parent trait was strongly inherited in F_2 . A few genes controlled the quantitative characters via additive gene action for days to flowering, plant height, stem diameter, leaf length, panicle length and diameter, also wet and dry panicle weight. Meanwhile, the leaf number and width, also grain weight per panicle were controlled by complementary epistasis. The wet and dry panicle weight had a significant positive correlation (0.9 and 0.95) with grain weight per panicle although there had low h^2_{bs} (0%). Panicle length and diameter could be used as indirect selection characters because of their significant positive correlation (0.67 and 0.7) to grain yield and high h^2_{bs} (90 and 80%).

Keywords: gene action, local variety, productivity, variance

ABSTRAK

Analisis genetik dan pola pewarisan pada karakter agronomi dan hasil diperlukan dalam merencanakan program pemuliaan dan seleksi yang akan dilakukan. Penelitian ini bertujuan untuk memperoleh informasi mengenai keragaan agronomi dan hasil, aksi gen, keragaman genetik dan heritabilitas pada populasi F_2 hasil persilangan varietas lokal dengan varietas berdaya hasil tinggi. Penelitian dilaksanakan dari bulan Agustus-November 2021 di Dramaga, Bogor. Material genetik yang digunakan adalah populasi F_2 , tetua betina dan jantan serta sepuluh genotipe pembandingan. Hasil penelitian menunjukkan karakter kualitatif pada F_2 cenderung lebih mirip tetua betina. Karakter hari berbunga, tinggi tanaman, diameter batang, panjang daun, panjang dan diameter malai, bobot malai basah dan kering dikendalikan oleh sedikit gen dengan aksi gen aditif. Karakter jumlah dan lebar daun serta bobot biji per malai dikendalikan oleh aksi gen epistasis komplementer. Bobot basah dan kering malai berkorelasi positif (0.9 dan 0.95) dengan bobot biji per malai dengan nilai h^2_{bs} (0%). Karakter panjang dan diameter malai dapat digunakan untuk seleksi tidak langsung karena kedua karakter berkorelasi positif (0.67 dan 0.7) dengan bobot biji per malai serta memiliki h^2_{bs} tinggi (90 dan 80%).

Kata kunci: aksi gen, keragaman, produktivitas, varietas lokal

INTRODUCTION

Sorghum is a major food crop that originated from Ethiopia, in northeastern Africa. In Indonesia, sorghum was introduced during the Dutch colonial period and is currently planted in 13 districts in East Nusa Tenggara and three districts in West Nusa Tenggara (Talanca and Andayani, 2013). Farmers in Indonesia have been cultivating several

local varieties of sorghum for generations. Sorghum Pulut varieties are known in East Nusa Tenggara. This local variety's superiority is its adaptability and quality, which can be used as genetic resources for crossbreeding parents and targeted for breeding targets. The use of local varieties is advantageous in sorghum breeding and contributes to the conservation and sustainable utilization of local genetic resources (Wulandari *et al.*, 2019). The disadvantage of this local variety is that it produces low yields, with an average productivity of less than 1 ton ha⁻¹. The low productivity of local varieties provides the bases for sorghum breeding.

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Production enhancement of local sorghum productivity could be done by crossing local sorghum with superior sorghum varieties that have a high yield. Self-pollination was performed on F_1 to obtain the F_2 generation which had the highest level of diversity to obtain information about the trait's variance, the number, and gene action that controlled the characters, heritability, and assess the indication of transgressive segregant (Sulistiyowati *et al.*, 2015). This information can be used as a basis for selection in a breeding program, whether selection can be made in the early or late generations (Kristantini *et al.*, 2019).

The Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University had been out plant breeding using the line \times tester crossing technique and resulting in a population of F_1 (Soraya 3 \times Pulut 3). The two parents used in the cross each had unique traits. Pulut 3 had a better taste quality due to its low amylose content but low productivity. Meanwhile, Soraya 3 was a promising parental line with high yield and protein content (Trikoesoemaningtyas, 2015). The objective of this research was to obtain information on the agronomic performance, gene action, and genetic variability of the F_2 population derived from crossing local and high-yielding varieties.

MATERIAL AND METHODS

The study was conducted at Leuwikopo Experimental Field, IPB University, Dramaga, Bogor, West Java, from August to November 2021. Post-Harvest handling was carried out at the Plant Breeding Laboratory Department of Agronomy and Horticulture, IPB University. The genetic material used were the F_2 population of Soraya 3 \times Pulut 3, female parent (Soraya 3), male parent (Pulut 3), and ten check genotypes (Numbu, Kawali, 867.032, CK 2, Butter Ainarup, Cantel Ketan, Keris, 431, ICSV 8801, Sorghum Putih). There were 600 plants of F_2 , 150 plants for each parent, and 30 plants for each check genotype planted at the same time and area. The parental and check genotypes were used to estimate the environmental variance, while the F_2 population expected phenotypic variance.

The experiment was carried out in a three-replication individual-based segregated population design (Jambormias and Riry, 2009). Parental and check genotypes in this research were used as a control genotype used to estimate the experimental error in a genetic experiment involving unreplicated F_2 individuals. The first stage of this study was land preparation and the application of manure (1 ton ha^{-1}) and dolomite (1.5 ton ha^{-1}) two weeks before planting. The seeds were planted at a spacing of 70 cm between plant rows and 10 cm between hills, with two seeds per hill. Urea, KCl, and SP36 fertilizers were applied at a dosage of 200 kg ha^{-1} , 100 kg ha^{-1} , and 100 kg ha^{-1} , respectively. Urea was applied twice; the first time two-thirds of the dose was applied one week after the plant (WAP), and the second time one-third of the dose was applied to four WAP, simultaneously with thinning and plowing. All agronomic and plant protection practices were consistent by adhering to the crop growth period to achieve the ideal crop stand.

All plants were observed for morphological, agronomic, and yield characteristics. The morphological characters observed in the tiller's presence, panicle density, and panicle shape (Elangovan *et al.*, 2014). On the other hand, flowering age, plant height, stem diameter, number of leaves, leaf length, leaf width, panicle length, panicle neck length, and panicle diameter were traits for the agronomic characters. Productivity characters such as panicle wet weight were measured after harvest, panicle dry weight was measured after 3 days of drying, and seed weight per panicle was measured after the milling process.

The data analyses carried out were: 1. analysis of the mean genotypes F_2 ($\bar{x} F_2$), P_1 ($\bar{x} P_1$), and P_2 ($\bar{x} P_2$) were tested using the t-student and visualized by a histogram, 2. estimation of gene action was tested through the chi-square test; 3. analysis of curve slope (skewness) and the sharpness of the curve (kurtosis); 4. estimation of mean and variance components; heritability and coefficient of genetic diversity or CGV; 5. Correlation between characters in the F_2 population. Correlation analysis was conducted to determine the relationship between variables using R with the agricolae package (de Mendiburu and Simon, 2015; de Mendiburu, 2021) and visualized using reshape2 and ggplot2 package (Wickham, 2016). The data were adjusted using SAS ODA and then it was analyzed using SPSS, Minitab, and Rstudio.

RESULTS AND DISCUSSION

Qualitative Traits on F_2 Sorghum Population

A qualitative character used to classify individuals into several categories that are clearly distinct from one another and it could distinguish clearly because it had discrete or discontinuous distribution (Nair, 2012). The qualitative character in this research was presented as a ratio in Table 1. Table 1 showed the appearance of the F_2 population was more similar to the female parent than the male parent. The panicle density character in the F_2 population showed a 100% compact panicle which is similar to the panicle density of Soraya 3. Meanwhile, the tiller's presence and panicle shape characters in the F_2 population had a 90% resemblance with the female parent. There is no segregation or ratio 1 for plant density character in the F_2 population indicating a single recessive gene controlled the characters (Uzun *et al.*, 2013). Sorghum cultivation focused on dense panicle density because it had a positive correlation with yield. Branching, inflorescence elongation, and spikelet fall affected the panicle density (Brown *et al.*, 2016). High-density and longer panicle branches could increase the number of grains, consequently, would increase the panicle grain weight (Sulistiyowati *et al.*, 2016).

The results of the Chi-square in Table 1 showed that the 1:3 segregation pattern on the presence of tiller character controlled by one major gene, where the presence of tiller was over-dominant to the absence of tillers in plants (Ritonga *et al.*, 2022). While the panicle shape character had a ratio of 15:1 indicating that the character was controlled

Table 1. Qualitative traits of parents and F₂ sorghum population

Characters	Character types	Observed percentage (%)			F ₂ test ratio	Interaction type	Chi-square test
		Soraya 3	Pulut 3	F ₂			
Present of tillers	Present	0	100	11.1	1:3	One dominant gene	0.46**
	Absent	100	0	88.9			
Panicle density	Compact	100	100	100.0	1	-	0.29ns
	Incompact	0	0	0.0			
Panicle shape	Middle with	100	0	91.7	15:1	Two duplicate dominant epistasis	0.36**
	Upside down pyramid	0	100	8.3			

by duplicated dominant epistasis. Epistasis is a type of pseudo deviation of Mendel's Law caused by interactions between dominant genes that overpower other dominant genes (Wibowo *et al.*, 2016).

Quantitative Traits on F₂ Sorghum Population

Figures 1C, 1G, and 1H depicted that the mean values of stem diameter, panicle length, and panicle neck length were lower than both parental. Meanwhile, the leaf length and width mean values in F₂ were higher than their parental (Figures 1E, 1F). The mean values of days to flowering, plant height, number of leaves, fresh panicle weight, dry panicle weight, and grain weight per panicle in the F₂ were all between the two parents' means (Figures 1A, 1B, 1D, 1J, 1K, 1L). The graph showed that the local sorghum yield had improved, as demonstrated by the mean value's enhancement of wet and dry panicle weight and grain weight per panicle (39.93, 33.43, and 34.03% respectively). The wide range of F₂ on fresh panicle weight (14.42-191.1 g), dry weight per panicle (13.7-166.7 g) and grain weight per panicle (2.9-163.2 g) indicated the possibility of high yielding segregant transgressive.

The influence of the female parents was suspected to play a role in the morphological and agronomic performances in F₂. Suwarno *et al.* (2016) discovered that female parents had a significant effect on the character of the number of rice tillers. It was also suspected that parental history affected the distribution of data in F₂. Soraya 3 results from a cross between B69 and Numbu, as proven by research by Rini *et al.* (2017), who found the Numbu variety to be good general combiners for grain yield. Maryono *et al.* (2019) discovered that the F₂ population of the B69 × Numbu cross had higher panicle weight and seed weight per panicle than the two parents.

Estimation of Gene Action Controlling Agronomic Characters and Yield of Sorghum

The skewness and kurtosis data could indicate the normal data distribution and gene action that controlled quantitative characters in the F₂ population. The normality of data distribution could determine from the skewness (S) value. A positive value indicated that the character was not only controlled by additive genes but also by complementary

epistasis. If the skewness value was negative indicated the influencing of additive gene action and duplicate epistasis (Roy, 2000). According to Roy (2000) and Jayaramachandran *et al.* (2010), the distribution of quantitative features in plants that lean to the left or right implies environmental impacts, genotype-environment interactions, gene linkages, and epistasis. Based on Jambormias *et al.* (2014) research, the results of skewness analysis and kurtosis values were interpreted to predict the action of the controlling gene for each character. The Z results showed that the days to flowering, plant height, stem diameter, leaf length, panicle length, panicle diameter, fresh panicle weight, and dry panicle weight were not significantly different (Table 2). This result implied that these traits had a normal distribution pattern and were affected by additive gene action. According to Griffiths *et al.* (2005), additive gene action could be interpreted as both parents contributing and being perceived to offspring.

The observed population's non-additive gene action characterized by additive and complimentary epistasis. Epistasis was the plant phenotype formed by the interaction of two or more genes from different loci. The complementary epistasis gene action affected the number of leaves, leaf width, and grain weight per panicle character. Complementary epistasis was a gene interaction in which the function of one gene is required by another gene in a metabolism (Roy, 2000; Griffiths *et al.*, 2005). According to Sulistyowati (2015), the presence of epistasis causes the fixation process of alleles to increase homozygosity to take longer. The skewness and kurtosis of the curve could also be utilized to estimate the gene action and the number of control genes. The positive kurtosis (leptokurtic) or negative (platykurtic) imply that the character is regulated by a few or many genes. The amount of regulating genes influences the breeding program difficulty (Roy, 2000).

Table 2 showed all of the traits controlled by a few genes. According to Xiao-Ping *et al.* (2011), leaf length performance is controlled by genes on chromosomes 6 and 10, resulting in an over-dominant effect based on research in two environments. Each of these genes contributed to leaf length diversity ranging from 7.60–20.20%, with the total level of gene contribution in the two experimental environments being 79.70 and 81.50%, respectively. Leaf width was determined by genes on chromosomes 1, 6, and 4 that act in dominant, partially dominant, or additive gene

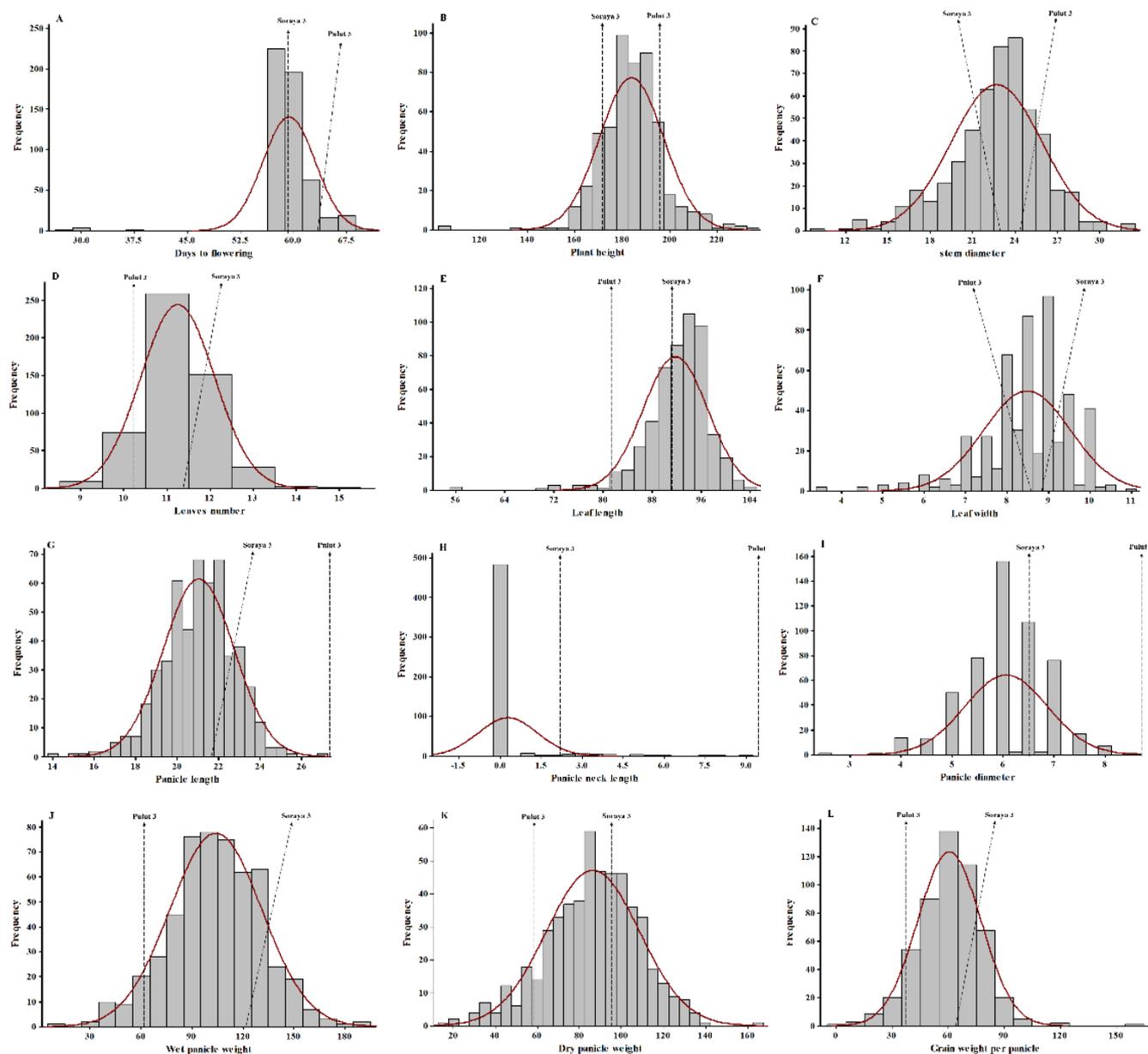


Figure 1. Quantitative data distribution in the sorghum F_2 population: (A) Days to flowering, (B) Plant height, (C) Stem diameter, (D) Leaves number, (E) Leaf length, (F) Leaf width, (G) Panicle length, (H) Panicle neck length, (I) Panicle diameter, (J) Fresh panicle weight, (K) Dry panicle weight, (L) Grain weight per panicle

action. Each of these genes contributed to the diversity of leaf width in the range of 9.60-20.80%, with the total level of gene contribution in the two experimental environments being 71.90% and 68.50%, respectively. According to Suwanto (2013), the wider the leaf, the more radiation can be intercepted for photosynthesis.

Estimation of Variance Components and Heritability

The component of variance estimation was used to determine the proportion of variation driven by genetic and environmental factors. The variance and standard deviation values in Table 3 were used to assess the level of diversity of the characters. If the variance value was twice as significant as the standard deviation, the tested characters have a high

degree of diversity (Sa'diyah *et al.*, 2013). Table 3 showed the diversity and range of plant height, fresh panicle weight, dry panicle weight, and grain weight per panicle character indicating an opportunity to obtain high-yielding transgressive segregants individuals.

The heritability value was used to estimate the level of selection progress and how much the characters can be improved. Selection would be effective if high heritability values were followed by high genetic progress (Maryono, 2019). Table 3 showed high broad-sense heritability values for stem diameter, leaf number, leaf width, panicle length, and panicle diameter characters. A high heritability value indicated that genetic factors outweigh the phenotype. High heritability values have an essential meaning in the effectiveness of selection, and selection can be made in the

Table 2. Skewness, kurtosis, gene action and number of controlling genes in the F₂ sorghum population

Characters	S	Z _s	SE _s	K	Z _k	SE _k	Gene action	Number of controlling genes
DTF	-3.25	-30.47ns	0.14	32.11	129.22**	0.21	Ad	A few
PH	-0.62	-5.84ns	0.14	5.00	28.28**	0.21	Ad	A few
SD	-0.33	-3.09ns	0.14	1.04	3.57**	0.21	Ad	A few
LN	0.27	2.69**	0.14	0.90	4.14**	0.21	EK	A few
LL	-2.01	-18.86ns	0.14	9.49	44.10**	0.21	Ad	A few
LW	-1.16	-10.88ns	0.14	124.02	12.73**	0.21	EK	A few
PL	-0.32	-3.03ns	0.14	25.54	3.68**	0.21	Ad	A few
PNL	3.08	28.96**	0.14	24.27	69.71**	0.21	EK	A few
PD	-0.35	-3.25ns	0.14	0.84	3.15**	0.21	Ad	A few
WPW	-0.06	-0.58ns	0.14	0.30	1.46ns	0.21	Ad	A few
DPW	-0.27	-2.55ns	0.14	0.34	1.61ns	0.21	Ad	A few
GWPP	0.23	2.12*	0.14	2.65	12.64**	0.21	EK	A few

Note: DTF = Days to flowering; PH = Plant height; SD = Stem diameter; LN = Leaves number; LL = Leaf length; LW = Leaf width; PL = Panicle length; PNL = Panicle neck length; PD = Panicle diameter; WPW = Fresh panicle weight; DPW = Dry panicle weight; GWPP = Grain weight per panicle; S = skewness; Z_s = statistic test for skewness; SE_s = standard error of skewness; K = kurtosis; Z_k = statistic test for kurtosis; SE_k = standard error for kurtosis; ** = significant at 1% level; * = significant at 5% level; ns = not significant for skewness and kurtosis test. Ad = Additive, EK = Complementary epistasis

early generations due to the low environmental influence on the appearance of these characters. Table 3 also showed Low heritability values for days to flowering, plant height, leaf length, panicle neck length, fresh panicle weight, dry panicle weight, and grain weight per panicle. According to Priyanto *et al.* (2018), a heritability value of 0% indicated environmental factors primarily cause phenotypic diversity. Grain weight per panicle is a popular plant productivity selection criterion. In this study, grain weight per panicle was affected by the action of complementary epistasis

genes, low broad-sense heritability, and low coefficient of genetic diversity. Thus, this character could not be used as a selection character in the early generation. It is better to do a direct selection on grain weight per panicle character in the late generation to accumulate additive genes first.

Correlation Between Agronomic and Yield Traits

Correlation analysis was used to estimate the pattern of relationships between agronomic characteristics and

Table 3. The estimated variance components and broad sense heritability values in the F₂ sorghum population

Characters	Variance	Std. Dev.	σ_p^2	σ_e^2	σ_g^2	h ² _{bs}	Criteria
DTF	15.71	3.96	6.56	82.94	-76.39	0.00	Low
PH	197.41	14.05	0.79	3,562.81	-3,562.02	0.00	Low
SD	11.73	3.43	119.64	46.84	72.79	0.61	High
LN	0.78	0.88	87,4617.75	2.35	874,615.4	1.00	High
LL	27.49	5.24	94.29	115.98	-21.68	0.00	Low
LW	1.12	1.06	6.64	1.92	4.72	0.71	High
PL	2.85	1.69	223.67	18.66	205	0.92	High
PNL	1.55	1.25	4.36	38.08	-33.72	0.00	Low
PD	0.67	0.82	22.31	4.50	17.81	0.80	High
WPW	736.07	27.13	106.84	939.58	-832.74	0.00	Low
DPW	495.23	22.25	4.46	616.01	-611.55	0.00	Low
GWPP	291.23	17.06	1.66	336.77	-335.11	0.00	Low

Note: DTF = Days to flowering; PH = Plant height; SD = Stem diameter; LN = Leaves number; LL = Leaf length; LW = Leaf width; PL = Panicle length; PNL = Panicle neck length; PD = Panicle diameter; WPW = Fresh panicle weight; DPW = Dry panicle weight; GWPP = Grain weight per panicle

yields. A positive coefficient value indicated that increasing character a will increase character b. The negative correlation value indicated that increasing character a reduces character b. (Saputra *et al.*, 2016). Figure 2 showed that there was a highly significant correlation between dry and fresh panicle weight and grain weight per panicle (0.95 and 0.9), followed by panicle diameter (0.7), panicle length (0.67), and stem diameter (0.48). Plant height and harvesting age were both adversely linked with grain weight per panicle. This proves that increasing panicle weight can increase plant productivity. As a result, one of these two characters could be used as a target character in the sorghum breeding process to improve productivity with additional consideration of heritability value. In this study, the wet and dry panicle weight and grain weight per panicle had a very low heritability value. So, the selection of this character was less effective when carried out on the F₂ population because the influence of environmental factors on these characters was quite large, allowing these characters to change when planted in different environments. The yield character was a complex character whose value was determined by many factors and is majorly influenced by the environment and it was recommended to choose the next generation for characters with low heritability (Ibrahim *et al.*, 2014).

Another technique is by using indirect selection or selecting characters that are closely related to wet and dry panicle weight and grain weight per panicle and have high heritability. Figure 2 showed that the panicle diameter and length character had a strong correlation with the yielding character and these characters had a high heritability. This demonstrates that the panicle ideotype correlates positively

with the yielding character and can be used as an indirect selection character. Wirnas *et al.* (2021) showed that when designing the ideotype of high-yielding sorghum requires a significant positive correlation of panicle diameter, stem diameter, leaf number, flag leaf area, leaf greenness, and green leaf percentage to the character of seed weight per panicle. Sulistyowati *et al.* (2016) discovered that panicle length was positively and very significantly correlated with panicle weight; the longer the panicle, the more branches of the panicle, and thus the weight of the panicle increased. The panicle neck length was inversely related to the seed weight per panicle, implying that the shorter the panicle neck, the higher the yield. According to Berenji *et al.* (2011), sorghum cultivation had been improved by requiring sorghum with a short or long panicle neck so that the panicles come out perfectly from the flag leaves, making harvesting easier and more efficient.

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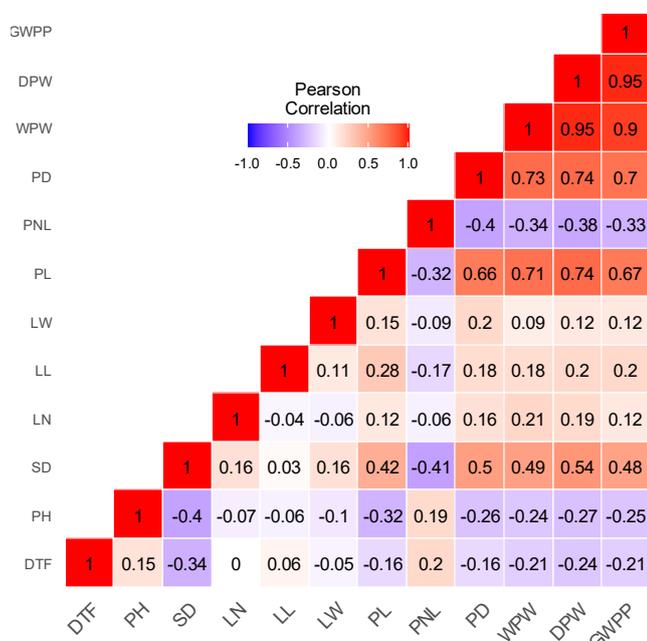


Figure 2. Correlation in F₂ Population

Note: DTF = Days to flowering; PH = Plant height; SD = Stem diameter; LN = Leaves number; LL = Leaf length; LW = Leaf width; PL = Panicle length; PNL = Panicle neck length; PD = Panicle diameter; WPW = Fresh panicle weight; DPW = Dry panicle weight; GWPP = Grain weight per panicle

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