GENETIC VARIATIONS IN SORGHUM SEGREGATING POPULATIONS BASED ON YIELD AND AMYLOSE CONTENT

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SUMMARY

The availability of sorghum varieties with good cooking quality will increase its use as a food source and for industrial needs. Amylose content is one of the biochemical characteristics that influence cooking quality. This study aimed to scrutinize the inheritance patterns of agronomical traits and amylose content in segregating the sorghum population. The field experiment was conducted from February to July 2022 at Cikarawang Experimental Field, IPB University, Bogor, Indonesia. The three F_2 populations, Pulut 3 × Kawali (372 plants), Pulut 3 × Soraya 3 IPB (340 plants), and Pulut 3 × PI-150-20A (363 plants) and their parental genotypes were evaluated. The three populations showed significant variations in agronomic traits. The panicle weight has a heritability value of 63% in Pulut 3 × Kawali, 36.7% in Pulut 3 × Soraya 3 IPB, and 59.8% in Pulut 3 × PI-150-20A. The waxy and non-waxy type segregation was apparent in F3 population seeds, with a 15:1 ratio in the Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A populations. Meanwhile, a 1:1 ratio was evident in the Pulut 3 × Kawali population. Selection could be conducted for waxy type at early generation followed by selection for yield at later generation.

Keywords: Sorghum (*S. bicolor* L.), cereal, breeding, genetic variations, segregating populations, starch, waxy

Key findings: The research produced information on genetic control of yield and sorghum (*S. bicolor* L.) type based on amylose content. This information would assist the food crop breeders in designing a breeding program for selection methods and criteria for developing high-yielding varieties and low-to-medium amylose content.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of many cereal crops serving diverse purposes, including food (Xiong *et al.,* 2019; Meena *et al.,* 2022), feed (Beretta *et al.,* 2021), and bioenergy sources (Suryaningsih and Irhas, 2014). Furthermore, grain sorghum is a viable alternative to rice, offering a comparable processing and cooking experience similar to *Oryza sativa*. The nutritional content of sorghum is equivalent to other cereals (Queiroz *et al.,* 2015). Then again, sorghum surpasses other cereals in its antioxidant compound content (Zhao *et al.,* 2022). Additionally, sorghum is a gluten-free food exhibiting a relatively lower glycemic index (Moraes *et al.,* 2015). Furthermore, sorghum can serve as a source of functional carbohydrates to mitigate the risk of degenerative diseases.

Apart from breeding for yield improvement (Wirnas *et al.*, 2021), pests, and disease resistance (Rifka *et al.*, 2020; Dinanty *et al.*, 2022), breeding for grain quality improvement has become an imperative breeding goal in sorghum. The diverse array of products derived from sorghum necessitates distinct grain characteristics, consequently influencing the crop ideotype. Particularly for human consumption, especially as a rice substitute, eating quality becomes a preference attribute for consumers. This quality encompasses stickiness, hardness, appearance, and taste, predominantly shaped by the physicochemical composition of the grain (Wang *et al.*, 2015; Li and Gilbert, 2018). One noteworthy physicochemical property is starch, consisting of amylose and amylopectin (James *et al.,* 2003). Grains with markedly low amylose content exhibit stickiness, moisture, and tenderness upon cooking, while those with intermediate levels manifest fluffiness, softness, moisture, and tenderness. In contrast, grains with high amylose content exhibit fluffiness, dryness, and firmness post-cooking (Arif *et al.,* 2013). Sorghum classification can consist of low amylose content percentage (<2%), medium (10%–20%), and high (>20%) (Puspitasari *et al.*, 2012).

Sorghum varieties with elevated amylose content are readily available, while those with medium (approximately 20%) amylose content remain scarce. Consequently, a need to develop high-yielding sorghum varieties with medium amylose content will address diverse consumer preferences. The extensive exploration of inheritance patterns of amylose content has progressed in cereals, such as rice (Kitara *et al.,* 2019) and maize (Zhang *et al.,* 2020). However, sorghum's quantitative and qualitative inheritance of amylose content attained limited attention. Therefore, a genetic study is crucial as an initial step to determine the breeding mode for amylose content and facilitate yield improvement.

Breeding initiatives pursued enhancing the amylose content in sorghum, employing germplasm characterization activities (Rachman *et al.*, 2022; Suroya *et al.*, 2023). The waxy allele examination in the sorghum germplasm of Indonesia reveals that the majority carries the wx^a and wx^c alleles. Among the 58 genotypes assessed, five landraces emerged as bearing the waxy alleles wx^a or wx^c, with amylose content varying between 5.6% and 23.3% (Suroya *et al.*, 2023). Nevertheless, these five genotypes demonstrate limited yield potential. Within breeding programs, these landraces can function as a genetic resource for enhancing amylose content by crossing them with high-yielding varieties, thus producing a segregating population characterized by elevated yield potential and amylose content.

Previous investigations have successfully acquired a segregating population from the hybridization of waxy and national (non-waxy) sorghum varieties. This work additionally conducted a genetic analysis of sorghum varieties, considering their amylose content and the heritability of agronomical traits within diverse sorghum populations to enhance the existing knowledge. The primary objective of this study is to scrutinize sorghum's agronomical traits and amylose content inheritance patterns.

MATERIALS AND METHODS

Genetic material and experimental site

The field experiment transpired from February to July 2022 at the Cikarawang Experimental Field and Plant Breeding Laboratory, IPB University, Bogor, West Java, Indonesia, situated at 175 masl. The study utilized three F_2 populations derived from the crosses Pulut 3 × Kawali, Pulut 3 × Soraya 3 IPB, and Pulut 3 × PI-150-20A, with four corresponding parental populations, Pulut 3, Kawali, Soraya 3 IPB, and PI-150-20A. Pulut 3 represents local Indonesian sorghum from Nusa Tenggara Barat Province; Soraya 3 is part of the IPB University breeding collection; Kawali is a released variety, and PI-150-20A is an accession from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The collective planting of these populations with their respective parents formed a cohesive experimental subset.

Procedure

Field experiment

The F_2 population planting with its parental lines in distinct plots had a 75 cm × 20 cm spacing, each accommodating one seed per planting hole. Dolomite application ensued a month before planting with a dose of 1 t ha^{-1}. Urea (45% N), KCl (60% K_2O), and SP36 (36% P_2O_5) are the fertilizers used with doses of 150 kg ha^{-1}, 100 kg ha^{-1}, and 100 kg ha^{-1}, respectively. One-third of the urea fertilizer treatment was a basal dose, with the 2/3 parts given four weeks after planting. All the recommended agronomic and plant protection methods proceeded accordingly. Thirty plants from each parent, selected as samples, encompassed all F_2 genotypes.

Harvesting materialized when the seeds had hardened, indicative of a black layer at the base of the seeds. Field observations, encompassing morphological, physiological, and agronomic traits, continued on yield and related characteristics. Thirty plants from each parent served as samples, with all F_2 genotypes.

Iodine staining

Sorghum grains sustained iodine staining based on a method described by Pedersen *et al*. (2004).

Data analysis

The data analysis to determine the mean values of each characteristic succeeded within the tested population. Gene action estimation for agronomic traits continued based on the F_2 distribution analysis, considering skewness and kurtosis values using the Statistical Tool for Agricultural Research (STAR) v2.0.1 developed by IRRI (Gulles *et al.,* 2014). The determination of heritability values and genetic coefficient of variation was according to: $\sigma_e^2 = (\sigma_{P1}^2 + \sigma_{P2}^2)/2$; $\sigma_p^2 = \sigma_{F2}^2$; $\sigma_g^2 = \sigma_p^2 - \sigma_e^2$; $h_{bs}^2 = \sigma_g^2/\sigma_p^2$; $GCV = \sigma_e^2/x$, with the variables representing: σ_g^2 = genotype variance; σ_e^2 = environmental variance; σ_p^2 = phenotype variance; h_{bs}^2 = broad-sense heritability; GCV = genotypic coefficient of variation; and x = general mean.

The sorghum seed type, characterized by amylose content—specifically low amylose content (waxy type) and high amylose content (non-waxy type)—underwent a Genetic Mendelian analysis. The proposed hypothesis suggests that the sorghum type is governable by genes located within the nucleus, involving two alleles, *Wx* and *wx*.

RESULTS AND DISCUSSION

Parental genotypes performance

This study used Pulut 3, Kawali, Soraya 3 IPB, and PI-150-20A as the parental lines and assessed agronomical traits to see a variation of each line compared with its F_2 populations. All parents, except for PI-150-20-A, have higher mean plant height values than the mean plant height of all parents. For stem diameter, both Pulut 3 and PI-150-20A have a stem diameter higher than all parents do. For leaf area, the mean value for all parents is 490.3, indicating that only Kawali and PI-150-20A have a leaf area higher than the mean of all

Table 1. The general mean value of each and four parental lines for agronomic traits.

Characteristics	Pulut 3	Kawali	Soraya 3 IPB	PI-150-20A	Mean
Plant height (cm)	169.4	178.2	163.8	124.7	159.0
Stem diameter (cm)	1.9	1.8	1.6	2.0	1.8
Leaf area (cm^2)	465.9	507.3	466.8	521.3	490.3
Leaf number	12.3	13.6	11	11.2	12.0
Panicle diameter (cm)	7.9	5.0	5.6	6.9	6.4
Panicle length (cm)	27.5	25.1	21.4	19.3	23.3
Panicle weight (g)	65.8	84.3	86.3	88.6	81.3
100-grain weight (g)	2.6	2.7	3.0	3.2	2.9

parents. On the leaf number, Kawali and Pulut 3 have leaf numbers of 13.6 and 12.3, respectively, both exceeding the mean of all parental plants (12). Regarding panicle diameter and length, Pulut 3 has higher mean values than the mean of all parents.

Meanwhile, Soraya 3 IPB and Kawali have lower panicle diameter length values. For mean panicle weight, all parents except for Pulut 3 have mean values higher than the mean of all parents (81.3 g), with PI-150-20A at 88.6 g, Soraya 3 IPB at 86.3 g, and Kawali at 84.3 g. In terms of 100-grain weight trait, all parents except for Pulut 3 (2.6 g) have mean values lower than the mean of all parents (2.9 g), namely, PI-150-20A (3.2 g), Soraya 3 IPB (3.0 g), and Kawali (2.7 g) (Table 1).

The parental characterization study conducted by Rachman *et al.* (2022) revealed only the parent Pulut 3 exhibited a waxy trait. Therefore, we selected Pulut 3 as the female parent and crossed it with the other three parents, each of which demonstrated superior agronomic traits compared with Pulut 3. The resulting F_1 progeny's subsequent subjection to self-pollination generated F_2 plants and F_3 seeds population for assessing segregation and trait inheritance about agronomic and waxy characteristics. The outcomes of these analyses follow:

Selecting parental lines is crucial for identifying suitable parents for commercial hybrid varieties and improving specific traits, such as higher yield and stress tolerance (Chung and Liao, 2022; Swarup *et al.*, 2020) and seed quality. Specifically, this study pursued sorghum with high productivity and waxy grain. This work discerned variations in

agronomical characteristic performance in three parental lines.

Plant height is an influential morphological and developmental phenotype that directly indicates overall plant growth and can serve as a crop breeding methods indicator (Joshi *et al.*, 2023). Manipulating plant height in sorghum and other cereals is essential for improving desirable traits, such as crop yield. Furthermore, in sorghum, plant height is a measurable trait directly affecting the yield (Wirnas *et al.*, 2021).

The parental line Pulut 3, which contributes to the waxy trait, has appeared to exhibit low yield potential. These findings suggest that the hybridization of Pulut 3 with other lines can yield recombinants and foster broader genetic diversity concerning amylose content and yield-related traits.

F_2 population performance

In this study, three F_2 populations used came from the hybridization involving Pulut 3 (a local variety) as the female parent and Kawali, Soraya 3 IPB, and PI-150-10-20A as the male parents. The highest plant height and broadest leaf area was the F_2 population of Pulut 3 × Kawali. The mean leaf numbers of the three populations were relatively close, ranging from 11.6 to 12.2, as well as, the stem diameters (2.0 and 2.3 cm) and 100-grain weights (2.4 and 2.6 g). The population with the largest mean panicle diameter was Pulut 3 × PI-150-20A, measuring 8.8 cm with a range of 2.9–15.7 cm. The highest mean panicle length was visible in the F_2 population of Pulut 3 × Soraya 3 IPB at 29.9 cm, with a range of 10.5–37 cm, followed by Pulut 3 × PI-150-20A at 29.5 cm with a range of 20–35.5 cm. The F_2 population

of Pulut 3 × Kawali exhibited the maximum mean and range for panicle weight at 88 g and 19.1–185 cm, respectively (Table 2).

In this study, the average measurements for plant height, leaf area, stem diameter, panicle length, and panicle weight within the F$_2$ population, with the fresh panicle weight and dry weight of Pulut 3 × Kawali, surpassed the mean values observed in both parental lines. In contrast, the mean value for leaf number is comparatively lower than that of both parental lines. Furthermore, intermediate mean values within the F$_2$ lie between those of the two parents, exemplified by panicle diameter and 100-grain weight (Table 2).

The F$_2$ generation resulting from the ~~crossbreeding~~ a cross of Pulut 3 × Soraya 3 IPB exhibited higher mean values in leaf area, stem diameter, panicle diameter, and panicle length than both parents did. However, the mean F$_2$ values for 100-grain weight were lower compared to both parents. The F$_2$ population traits, such as plant height, leaf number, neck panicle length, fresh panicle weight, and dry panicle weight, were within the values observed for both parents (Table 2).

In contrast, the F$_2$ population resulting from the cross of Pulut 3 × PI-150-20A displayed mean F$_2$ values within the range observed for the parents for leaf number, panicle diameter, neck panicle length, fresh panicle weight, and dry panicle weight. Mean F$_2$ values exceeding those of both parents emerged for plant height, stem diameter, and panicle length, while leaf area, fresh panicle weight, and 100-grain weight occurred to be lower than the values observed for both parents (Table 2).

Table 2. F$_2$ populations' mean of agronomic characteristics.

Characteristics	Mean		STDV	F$_2$ population range		
Pulut 3 × Kawali						
Plant height (cm)	198.3	±	47.1	89.0	–	349.7
Leaf area (cm^2)	545.5	±	124.9	199.1	–	888.3
Leaf number	11.6	±	1.4	6.0	–	17.0
Stem diameter (cm)	2.0	±	0.4	0.9	–	3.0
Panicle diameter (cm)	6.8	±	1.5	2.3	–	11.4
Panicle length (cm)	28.6	±	3.5	14.2	–	39.0
Panicle weight (g)	88.0	±	30.8	19.1	–	185.0
100-grain weight (g)	2.6	±	0.4	1.2	–	3.8
Pulut 3 × Soraya 3 IPB						
Plant height (cm)	167.5	±	28.2	103.0	–	330.0
Leaf area (cm^2)	473.7	±	113.0	166.7	–	808.3
Leaf number	12.2	±	1.3	7.0	–	15.0
Stem diameter (cm)	2.0	±	0.5	0.7	–	3.09
Panicle diameter (cm)	7.5	±	1.9	1.0	–	12.7
Panicle length (cm)	29.9	±	3.0	10.5	–	37.0
Panicle weight (g)	67.1	±	25.9	8.3	–	215.8
100-grain weight (g)	2.4	±	0.4	1.1	–	3.7
Pulut 3 × PI-150-20A						
Plant height (cm)	171.3	±	30.8	89.2	–	298.0
Leaf area (cm^2)	516.1	±	98.5	185.3	–	796.1
Leaf number	12.2	±	1.2	9.0	–	15.0
Stem diameter (cm)	2.3	±	0.5	0.7	–	3.5
Panicle diameter (cm)	8.8	±	1.6	2.9	–	15.7
Panicle length (cm)	29.5	±	2.5	20.0	–	35.5
Panicle weight (g)	81.6	±	24.4	11.3	–	185.2
100-grain weight (g)	2.6	±	0.4	1.4	–	3.5

Studying the F_2 population provides valuable insights into genetic and phenotypic variability, genetic control, , aiding in developing improved crop varieties with desirable agronomical traits. The higher mean value of F_2 than its parents indicate the presence of F_2 segregants with phenotypic traits superior to both parents. This phenomenon due to the segregation and recombination of genes resulting from the crossbreeding of the two parents, leading to the emergence of phenotypes distinct from the parents. These individuals become transgressive segregants. The occurrence of transgressive segregants in the F_2 population is due to the control of additive gene action

Inheritance of agronomical traits

Estimating gene actions on traits can proceed by examining the distribution of values within a segregating population using two parameters-skewness and kurtosis. Skewness values depict gene actions, whereas kurtosis values describe the number of genes controlling a particular trait.

Plant height and panicle weight control come from complementary epistasis in the F_2 population of Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A. However, in the Pulut 3 × Kawali population, the action of dominant genes controls them. Leaf area and plant diameter in Pulut 3 × Kawali and Pulut 3 × Soraya 3 IPB results from the action of additive genes, whereas in Pulut 3 × PI-150-20A, duplicate epistasis dominated leaf area and panicle diameter by complementary epistasis. The leaf number trait in Pulut 3 × Kawali, Pulut 3 × Soraya 3 IPB, and Pulut 3 × PI-150-20A is effected by complementary epistasis, duplicate epistasis, and additive genes, respectively. Additive genes controlled stem diameter in Pulut 3 × Kawali, while dominant ones in Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A. Panicle length in the F_2 population of Pulut 3 × Kawali gained dominance by additive epistasis, with the other two F_2 populations, Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A, controlled by duplicate epistasis gene action. For the 100-grain weight trait, a dominant gene action controls the F_2 population of Pulut 3 × Kawali, while additive gene action controls the F_2 populations of Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A (Table 3).

Additive gene action plays a role in leaf area and panicle diameter traits in the Pulut 3 × Kawali and Pulut 3 × Soraya 3 IPB populations while influencing the 100-grain weight trait in the Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A populations (Table 3). Additive gene action implies that the observed phenotypes exhibit a high level of heritability in their progeny (Daudi *et al*., 2021).

Table 3. Gene action, heritability, and coefficient of genotypic variance of agronomic characteristics on three F_2 sorghum populations.

Traits	Pulut 3 × Kawali			Pulut 3 × Soraya 3 IPB			Pulut 3 × PI-150-20A		
	Gene action	h_{bs}^2 (%)	GCV (%)	Gene action	h_{bs}^2 (%)	GCV (%)	Gene action	h_{bs}^2 (%)	GCV (%)
Plant height	D	91.1	22.7	CE	72.2	14.3	CE	80.4	16.1
Leaf area	A	70.0	19.1	A	59.5	18.4	DE	37.6	11.7
Leaf number	CE	42.8	7.8	DE	31.1	6.0	A	49.6	6.7
Stem diameter	A	36.6	10.8	D	72.7	20.2	D	67.8	12.9
Panicle diameter	A	50.1	15.8	A	60.1	19.7	CE	81.2	16.4
Panicle length	AE	70.2	10.1	DE	56.6	7.5	DE	8.40	2.4
Panicle weight	D	63.3	27.9	CE	36.7	23.4	CE	59.8	23.1
100-grain weight	D	70.9	13.6	A	60.4	12.5	A	74.3	11.6

Note: h_{bs}^2 = broad-sense heritability; GCV = genotypic coefficient of variation; A = additive; D = dominant; CE = complementary epistasis; AE = additive epistasis; DE = duplicate epistasis.

Non-additive gene actions, such as dominance and epistasis, are crucial in nearly all observed traits within the three tested populations (Table 3). Non-additive gene actions result in higher mean phenotypic values in the F_2 generation than the parents; however, a significant portion of these traits may be uninherited in the progeny (Trikoesoemaningtyas *et al*., 2017). Dominant gene actions are particularly influential in the Pulut 3 × Kawali population's several agronomic attributes, including plant height, neck panicle weight, dry panicle weight, and 100-grain weight. The dominance effect on these traits indicates that early-generation selection is less effective and must defer to later generations (Salem *et al*., 2020).

Epistatic gene actions control numerous characters in the Pulut 3 × PI-150-20A population. The types of epistatic gene actions identified in this study include additive, complementary, and duplicate epistasis. Complementary epistasis can enhance heterosis, increasing diversity, while duplicate epistasis has the opposite effect (Yudilastari *et al*., 2018). Noteworthily, favorable trait performance manifestation may not necessarily reappear in subsequent generations when epistatic gene actions govern these traits (Maryono *et al*., 2019). Most sorghum agronomic traits in three populations exhibit moderate to high broad-sense heritability, except for panicle length in the Pulut 3 × PI-150-20A population. High broad-sense heritability values indicate that genetic factors controlled a significant proportion of the observed phenotypic variation.

Another parameter for assessing the genetic diversity level of a population for a given trait is the genetic coefficient of variance (GCV). The GCV values for several sorghum agronomic traits in the three populations (Table 3) attained categories as moderate to high for leaf number in all three populations and panicle length in the Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A populations.

The traits, such as plant height, panicle diameter, and 100-grain weight, manifest elevated heritability across all F_2 populations, displaying values spanning from 72.2% to 91.1%, 50.1% to 81.2%, and 60.4% to 74.3%, respectively. Leaf area and panicle length traits exhibit heightened heritability, specifically in Pulut 3 × Kawali and Pulut 3 × Soraya 3 IPB populations, recording values of 59.5% and 70% for leaf area and 56.6% and 70.2% for panicle length. Similarly, panicle weight demonstrates notable heritability in Pulut 3 × Kawali and Pulut 3 × PI-150-20A populations, registering values of 70.2% and 56.6%. Meanwhile, the stem diameter trait displays elevated heritability in F_2 populations of Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A, with values of 72.7% and 67.8%, respectively (Table 3).

Inheritance of amylose content

This study discloses the variability of amylose content in the parental cross seeds and F_3 population (Figure 1). Waxy sorghum was distinct with a brown-reddish endosperm through iodine staining, as shown in the Pulut 3. Meanwhile, non-waxy sorghum exhibited a dark blue endosperm in Kawali, Soraya 3 IPB, and PI-150-20A (Figure 1A). Figure 1B showed that the F_3 population seed had diverse waxy and non-waxy potentials.

Figure 1. Sorghum amylose content variation based on iodine staining. A. parental seeds; B. F_3 population seeds.

The waxy and non-waxy type segregation was evident in F_3 seeds, with a 15:1 ratio in the Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A populations, with a 1:1 ratio identified in the Pulut 3 × Kawali population (Table 4). Reports of a segregation ratio of 1:1 for waxy and non-waxy traits also came from Boyles *et al.* (2017) in RILs sorghum populations. The observed differences in Mendelian ratios among the three populations may be due to genotype variations in the Pulut 3 population. Based on Mendelian genetic analysis, we predicted a one-pair dominant gene controlled Pulut 3 × Kawali, with Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A regulated by two pairs with dominant duplicate genes.

Amylose is one of the polymers composing starch in the endosperm. The amylose content in the endosperm of seeds is paramount in determining grain-eating quality (Sompong *et al.*, 2011). Rapid qualitative assessment of amylose content can be detected through the iodine staining approach. The selection of the staining method was based on its simplicity and cost-effectiveness, rendering it highly suitable for initial screening or large-scale population studies.

The analysis of the inheritance of the waxy trait in the data obtained, based on the method developed by Pedersen *et al.* (2004), is referred to as Mendelian genetic analysis. Several assumptions underlying Mendelian genetic analysis include the control of the waxy trait by a pair of genes, namely, the Wx and wx alleles, where the Wx allele is dominant to wx, with both alleles found on the nucleus chromosome. The production of amylose in grain starch, identified as a Mendelian inherited trait (Boyles *et al.*, 2017), has regulations of the well-characterized waxy (Wx) gene situated on chromosome 10, encoding granule-bound starch synthase I (McIntyre *et al.*, 2008).

The genotype classification of Pulut 3 as a waxy landrace with an average amylose content of 6.30%. Various tests on the amylose content of Pulut have revealed divergent results, assumably due to the inherent heterogeneity within the landrace population. Consequently, the waxy genotype in Pulut 3 may manifest in either Wxwx or wxwx forms, as indicated by staining tests revealing a reddish-brown coloration in the endosperm.

The research findings revealed that the waxy trait inheritance comes from the female parent. F_3 genotypes classified as waxy resulted in 50% or more for all three populations tested. Given the maternal effect, the waxy genotype can probably possess the following genotypes: WxWxwx, Wxwxwx, or wxwxwx. Amylose synthesis gains control from the Wx locus in cereal plants, which will manifest in the endosperm (Denyer *et al.*, 2001). The presence of maternal effect in the inheritance of the waxy trait in sorghum constitutes crucial information for the enhancement of amylose content in sorghum. The monogenic nature of the waxy trait facilitates its straightforward introgression, particularly upon the development of elite, high-starch genotypes (Boyles *et al.*, 2017).

The amylose biosynthesis regulation came from the granule-bound starch synthase (GBSS) enzyme, whereas the starch-branching enzyme (SBE) controlled amylopectin (Huang *et al.*, 2021). The phenotypic characteristics of the stained endosperm implied the presence of these two polymers, thus indicating the genetic control of traits by two gene pairs in this study.

The waxy gene is a recessive gene resulting from a mutation of the gene encoding granule-bound synthase I (GBSS I) enzyme. This gene exists on chromosome number 10. GBSS I is crucial in amylose synthesis, and mutations in this gene lead to the loss of amylose synthesis capability (McIntyre *et al.*, 2008). Based on the mutational positions of the GBSS I gene, four types of mutant alleles have appeared, namely, wx^a, wx^b, wx^c, and wx^d (Lu *et al.*, 2013).

CONCLUSIONS

The results showed a variation caused by genetic factors in agronomic characteristics in the three populations tested. The panicle weight has a heritability value of 63% in Pulut 3 × Kawali, 36.7% in Pulut 3 × Soraya 3 IPB, and 59.8% in Pulut 3 × PI-150-20A. The waxy

and non-waxy type segregation was evident in F_3 population seeds, with a 15:1 ratio in the Pulut 3 × Soraya 3 IPB and Pulut 3 × PI-150-20A populations, while a 1:1 ratio was distinct in the Pulut 3 × Kawali population.

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REFERENCES

Arif AB, Budiyanto A, Hoerudin (2013). Nilai Indeks Glikemik Produk Pangan Dan Faktor-Faktor Yang Memengaruhinya. *J Litbang Pertan*. 32(3): 91–99. (In Indonesian).

Beretta V, Simeone A, Franco J, Bentancur O, Novac M, Panizza V, Rodríguez MV (2021). Using sorghum dry distillers' grains plus soluble in sorghum-based finishing diets: Feed utilization, cattle performance, and carcass traits. *Anim. Feed Sci. Technol.* 271: 114731. https://doi.org/10.1016/j.anifeedsci.2020.114731.

Boyles RE, Pfeiffer BK, Cooper EA, Rauh BL, Zielinski KJ, Myers MT, Brenton Z, Rooney WL, Kresovich S (2017). Genetic dissection of sorghum grain quality traits using diverse and segregating populations. *Theor*. *Appl. Genet*. 130: 697–716. https://doi.org/10.1007/s00122-016-2844-6.

Chung PY, Liao TC (2022). Selection of parental lines for plant breeding via genomic prediction. *Front. Plant Sci*. 13: 934767. https://doi.org/10.3389/fpls.2002.934767.

Daudi H, Shimelis H, Mathew I, Rathore A, Ojiewo CO (2021). Combining ability and gene action controlling rust resistance in groundnut (*Arachis hypogaea* L.). *Sci. Rep*. 11: 16513. https://doi.org/10.1038/s41598-021-96079-z.

Denyer K, Johnson P, Zeeman S, Smith AM (2001). The control of amylose synthesis. *J. Plant Physiol*. 158(4): 479–487. https://doi.org/10.1078/0176-1617-00360.

Dinanty F, Wirnas D, Trikoesoemaningtyas, Nawangsih AA (2022). Diversity of leaf rust resistance in Indonesian sorghum genetic resources. *Biodiversitas.* 23(5):2570–2579. https:// doi: 10.13057/biodiv/d230538.

Gulles AA, Bartolome VI, Morantte R, Nora LA (2014). Randomization and analysis of data using STAR (Statistical Tool for Agricultural Research). *Phillip. J. Crop Sci*. 39: 137.

Huang L, Tan H, Zhang C, Li Q, Liu Q (2021). Starch biosynthesis in cereal endosperms: An updated review over the last decade. *Plant Comm*. 2: 100237. https://doi.org/10.1016/j.xplc.2021.100237.

James MG, Denyer K, Myers AM (2003). Starch synthesis in the cereal endosperm. *Curr. Opin Plant Biol*. 6(3): 215–222. https://doi.org/10.1016/s1369-5266(03)00042-6.

Joshi BK, Ghimire KH, Neupane SP, Gauchan D, Mengistu DK (2023). Approach and advantages of increased crop genetic diversity in the fields. *Diversity*. 15(5): 603. https://doi.orh/10.3390/d15050603.

Kitara IO, Lamo JR, Gibson P, Rubaihayo P (2019). Amylose content and grain appearance traits in rice genotypes. *Afr. Crop Sci. J*. 27(3): 501–513. https://doi.org/10.4314/acsj.v27i3.12.

Li H, Gilbert RG (2018). Starch molecular structure: The basis for an improved understanding of cooked rice texture. *Carbohydr. Polym.* 195: 9-17. https://doi.org/10.1016/j.carbpol.2018.04.065.

Lu Y, Zhao G, Li Y, Fan J, Ding G, Zhao J, Ni X, Xu Y, Wang W (2013). Identification of two novel waxy alleles and development of their molecular markers in sorghum. *Genome*. 56(5):283–288.
https://doi.org/10.1139/gen-2013-0047.

Maryono MY, Trikoesoemaningtyas, Wirnas D, Human S (2019). Genetic analysis and transgressive segregant selection of F_2 sorghum population derived from B69 × Numbu and B69 × Kawali crosses. *J. Agron. Indonesia.* 47(2):163–170. https://doi.org/10.24831/jai.v47i2.24991. (In Indonesian).

McIntyre CL, Drenth J, Gonzalez N, Henzell RG, Jordan DR (2008). Molecular characterization of the waxy locus in sorghum. *Genome*. 51(7):524–533. https://doi.org/10.1139/G08-035.

Meena K, Visarada KBRS, Meena DK (2022). *Sorghum bicolor* (L.) Moench a multifarious crop-fodder to therapeutic potential and biotechnological applications: A future food for the millenium. *Future Foods*. 6:100188. https://doi.org/10.1016/j.fufo.2022.100188.

Moraes EA, da Silva Marineli R, Lenquiste SA, Steel CJ, de Menezes CB, Queiroz VAV, Júnior MRM (2015). Sorghum flour fractions: Correlations among polysaccharides,

phenolic compounds, antioxidant activity, and glycemic index. *Food Chem*. 180: 116–123. https://doi.org/10.1016/j.foodchem.2015.02.023.

Pedersen JF, Bean SR, Funnell DL, Graybosch RA (2004). Rapid iodine staining techniques for identifying the waxy phenotype in sorghum grain and waxy genotype in sorghum pollen. *Crop Sci*. 44(3):764–767. https://doi.org/10.2135/cropsci2004.7640.

Puspitasari W, Human S, Wirnas D, Trikoesoemaningtyas (2012). Evaluating genetic variability of sorghum mutant lines tolerant to acid soil. *Atom Indonesia*. 38 (2) 83–88.

Queiroz VAV, da Silva CS, de Menezes CB, Schaffert RE, Guimarães FFM, Guimarães LJM, de Oliveira Guimarães PE, Tardina FD (2015). Nutritional composition of sorghum (*Sorghum bicolor* [L.] Moench) genotypes cultivated without and with water stress. *J. Cereal Sci*. 65: 103–111. https://doi.org/10.1016/j.jcs.2015.06.018.

Rachman F, Trikoesoemaningtyas, Wirnas D, Reflinur (2022). Estimation of genetic parameters and heterosis through line × tester crosses of national sorghum varieties and local Indonesian cultivars. *Biodiversitas*. 23(3):1588–1597. https://doi.org/10.13057/biodiv/d230349.

Rifka, Wirnas D, Trikoesoemaningtyas (2020). Genotype by environment interaction affecting leaf rust resistance in sorghum. *SABRAO J. Breed. Genet*. 52 (1) 75–90.

Salem TSG, Rabie HA, Mowafy SAE, Eissa AEM, Mansour E (2020). Combining ability and genetic components of Egyptian cotton for earliness, yield, and fiber quality traits. *SABRAO J. Breed. Genet*. 52(4):369–389.

Sompong R, Siebenhamdl-Ehn S, Linberger-Martin G, Berghofer E (2011). Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China, and Sri Lanka. *Food Chem*. 124: 132–140. https://doi.org/10.1016/j.foodchem.2010.05.115.

Suroya LF, Wirnas D, Trikoesoemaningtyas, Reflinur (2023). Identification of waxy genotype in sorghum genetic resources using waxy gene-based markers and iodine staining methods. *Aust. J. Crop Sci.* 17(2): 190–197. https://doi.org/10.21475/ajcs.23.17.02.p3784.

Suryaningsih R, Irhas (2014). Bioenergy plants in Indonesia: Sorghum for producing bioethanol as an alternative energy substitute for fossil fuels. *Energy Procedia*, 47: 211–216. https://doi.org/10.1016/j.egypro.2014.01.216.

Swarup S, Cargill EJ, Crosby K, Flagel L, Kniskern J, Glenn KC (2020). Genetic diversity is indispensable for plant breeding to improve crops. *Crop Sci*. 61(2): 839–852. https://doi.org/10.1002/csc2.20377.

Trikoesoemaningtyas, Wirnas D, Saragih EL, Rini EP, Sari M, Marwiyah S, Sopandie D (2017). Genetic control of morphology and agronomic characters in three sorghum (*Sorghum bicolor* [L.] Moench) populations. *J. Agron. Indonesia.* 45(3):285–291. https://doi.org/10.24831/jai.v45i3.18387. (In Indonesian).

Wang K, Wambugu PW, Zhang B, Wu AC, Henry RJ, Gilbert RG (2015). The biosynthesis, structure, and gelatinization properties of starches from wild and cultivated African rice species (*Oryza barthii* and *Oryza glaberrima*). *Carbohydr. Polym*. 129: 92–100. https://doi.org/10.1016/j.carbpol.2015.04.035.

Wirnas D, Oktanti N, Rahmi HN, Andriani D, Faturrahman, Rini EP, Marwiyah S, Trikoesoemaningtyas, Sopandie D (2021). Genetic analysis for designing an ideotype of high-yielding sorghum based on existing lines performance. *Biodiversitas*. 22(12):5286–5292. https://doi.org/10.13057/biodiv/d221208.

Xiong Y, Zhang P, Warner RD, Fang Z (2019). Sorghum grain: From genotype, nutrition, and phenolic profile to its health benefits and food applications. *Compr. Rev. Food Sci. Food Saf.* 18(6): 2025–2046. https://doi.org/10.1111/1541-4337.12506.

Yudilastari T, Syukur M, Sobir (2018). Pewarisan karakter hasil dan komponen hasil pada dua populasi persilangan cabai rawit hijau (*Capsicum annuum* L.). *J. Agron. Indonesia.* 46(3): 283–289. https://doi.org/10.24831/jai.v46i3.21534. (In Indonesian).

Zhang X, Gao X, Li Z, Xu L, Li Y, Zhang R, Xue J, Guo D (2020). The effect of amylose on kernel phenotypic characteristics, starch-related gene expression, and amylose inheritance in naturally mutated high-amylose maize. *J. Integ. Agric*. 19(6): 1554–1564. https://doi.org/10.1016/S2095-3119(19)62779-6.

Zhao Y, Zhai G, Li X, Tao H, Li L, He Y, Zhang X, Wang F, Hong G, Zhu Y (2022). Metabolomics reveals nutritional diversity among six coarse cereals and antioxidant activity analysis of grain sorghum and sweet sorghum. *Antioxidants* 11: 1984. https://doi.org/10.3390/antiox11101984.