GENETIC STUDY OF AMYLOSE CONTENT AND YIELD-RELATED TRAITS IN SORGHUM GERMPLASM

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SUMMARY

In the breeding program for high-yielding sorghum, grain quality improvement needs high consideration to meet consumer demands. Thirty sorghum genotypes underwent genetic variation evaluation, with 13 undergoing assessment for genotype by environment effects on the amylose content. The research proceeded in three Indonesian provinces from June–December 2022. The results showed genetic variations among the sorghum genotypes for amylose content and yield traits with a high and medium heritability for yield traits and amylose content, respectively. The genotype Demak 4 had the lowest amylose content (8.57%), and 10 genotypes with amylose content had a range of 10% to less than 20% (Keris M-3, 431, CK.2, Rumbia, Pulut 3, Keris, Gadam Human, 867.032, Lokal Bima 3, and Cantel Ketan), and 19 genotypes showed amylose content higher than 20%. No correlation between amylose content and yield exists. The analysis of combined variance showed that genotypes had a highly significant effect; however, location and the interaction of genotype and location had no significant influence on the amylose content of sorghum. Genotypes Pulut 3 and CK.2 consistently received a heterowaxy classification, with Demak 4 classified as waxy sorghum by growing in three different environments. This data would benefit the selection of promising genotypes for crossing and designing a breeding program to develop high-yield cultivars with low and medium amylose content.

Keywords: Amylopectin, GE interaction, rice, simultaneous selection, quality

Key findings: The information produced by this research would help breeders select parental candidates in a breeding program for developing high-yielding cultivars with low or medium amylose content. The recommended genotypes for sources of genes controlling amylose content are Demak 4, Pulut 3, 431, CK 2, Lokal Bima 3, and Cantel Ketan.

INTRODUCTION

Starch is highly significant as a primary carbohydrate source in the human diet, constituting over half of our total carbohydrate consumption. Starch consists of two types of polymers, namely, amylose and amylopectin, stored in storage organs, such as rhizome, tuber, and grain. Low amylose grains of waxy sorghum have a high economic value in the food and bioenergy industries because of their increased starch and protein digestibility compared with non-waxy type sorghum grains (Walker and Lichtenwalner, 1977; Lu *et al.*, 2013; Mezgebe *et al.*, 2020).

The amylose of starch is primarily under genetic control (Karper, 1933; Yerka *et al.*, 2016). Sorghum classification comprises three groups based on the amylose content: waxy (<2%), heterowaxy (10%–20%), and non-waxy (>20%) (Rooney and Serna-Saldivar 2000; Puspitasari *et al.*, 2012) representing low, medium, and high amylose content, respectively. The waxy trait of sorghum is an inheritance from a simple Mendelian recessive gene (Karper, 1933). The amylose content of sorghum grain depends on the dose of a recessive gene (wx) (Sang *et al.*, 2008; Guzmán *et al.*, 2012). The Wx gene encodes the granule-bound starch synthase (GBSS) enzyme, synthesizing amylose within starch granules.

Waxy or heterowaxy sorghum has been a widely used food source (Cho *et al.*, 2015), while the non-waxy/low amylose content type generally serves as feed (Suarni, 2016). A study showed the testing of several cultivars, where panelists preferred rice from sorghum due to a lower amylose content (Budijanto and Yulianti, 2012). The physical configurations and ratio of amylose and amylopectin are the main factors in determining its function as a food or food ingredient source (Yerka *et al.*, 2016; Suarni, 2016; Htet *et al.*, 2022).

Sorghum cultivars grown in Indonesia have an amylose content of more than 23%, causing rice to be coarse in taste (Budijanto and Yulianti, 2012; Suarni, 2016; Avif and Oktaviana, 2021). The waxy sorghum cultivars are still available to local cultivars (Suroya *et al.*, 2023), which have a low yield potential and resistance to pests and diseases, hence hard to commercialize. It is necessary to improve the cooking quality by reducing the amylose content in sorghum grains of existing cultivars to increase its consumer acceptance as a food source. Breeding programs focusing on cooking quality enhancement require support with the availability of information on genetic diversity and the interaction of genotype × environment on amylose content and the interaction between amylose content and yield potential.

The study of the genetic diversity of crop species can use morphological, biochemical, and molecular markers (Cho *et al.*, 2015; Enyew *et al.*, 2022; Suroya *et al.*, 2023). Initial studies on 58 accessions using molecular markers showed that the national cultivars tested belonged to the non-waxy type. Two accessions were heterozygous for the wx^a allele, and 22 were heterozygous for the wx^c allele from the 58 accessions tested (Suroya *et al.*, 2023). The results of the staining test on seeds showed that only five accessions attained waxy classification of the 58.

Genetic studies, including the G×E effect on a crop, are crucial for effective germplasm management, utilization, and breeding strategy (Roy, 2000; Enyew *et al.*, 2022). Information about genetic diversity, the G×E effect on amylose content in sorghum grain, its correlation with yield potential in sorghum germplasm, and the GE effect of quality traits of sorghum is unavailable. This information is highly beneficial for breeders to develop sorghum cultivars, especially providing information on selecting parents as gene sources and efficient breeding strategies for

boosting amylose content and yield potential in sorghum. The research aims to study the diversity of agronomic traits and amylose content and their correlation with yield potential and G × E effect on the amylose content.

MATERIALS AND METHODS

The experiment consists of 1). The analysis of variability of sorghum types based on iodine staining and amylose content in sorghum germplasm, and 2). The scrutiny of the effect of genetic and environmental interactions on the amylose content of sorghum. A brief description of each location is available in Table 1.

Genetic material and experimental site

Experiment 1 transpired from June to October 2022 at the Agriculture Research Center for Biotechnology and Genetic Resources, Bogor, West Java, Indonesia. Laboratory work for iodine staining happened at the Plant Breeding Laboratory of the Department of Agronomy and Horticulture, IPB University. The postharvest laboratory of the Ministry of Agriculture analyzed the amylose content. The genetic materials used consisted of six check cultivars and 24 sorghum germplasm from the collection of the Agriculture Gene Bank, the Ministry of Agriculture. The accessions included 13 introduced lines, 12 landraces, and five national breeding lines (Table 2). These accessions' selection relied on the results of iodine staining of the previous research. Growing all genetic materials was under an augmented randomized complete block design (RCBD).

Experiment 2 ensued in July-November 2023 in three locations, namely, Bogor Regency (West Java Province), Natar Regency (Lampung Province), and Maros Regency (South Sulawesi Province). The genetic materials used were 13 genotypes with three replications nested within locations following the RCBD design.

Field experiment

The field experiment had the same procedures for experiments 1 and 2. The sowing of seeds had a spacing of 70 cm between rows and 15 cm between hills, with three seeds per hill. Two weeks after planting, reducing the number of plants continued to one per hill through thinning. Dolomite application progressed a month before planting with a dose of 1 t ha^{-1}. Application of Urea, KCl, and SP36 fertilizers had dosages of 150, 100, and 100 kg ha^{-1}, respectively. The 1/3 dose of Urea fertilizer application became a basal dose, and applying the 2/3 parts followed four weeks after planting. The study followed all recommended agronomic and plant protection practices, conducting field observation on agronomic characteristics.

Analysis of iodine staining and amylose content

Iodine staining of sorghum grains proceeded based on the staining method described by Pedersen *et al*. (2004). The amylose content estimation of the starch was according to SNI 6128:2008 using the spectrophotometry method (BSN, 2008).

Data analysis

The data analysis included means for each trait, as corrected by the existing effect of different growing environments. The adjusted means calculation used formulas by Sharma (2006). Data assessment with analysis of variances (ANOVA) used Rstudio v1.4.1106 with the Agricolae package, with the correlation between variables observed (de Mendiburu and Simon, 2015). Partitioning the expected mean squares helped estimate the components of variance based on the augmented design model.

Table 1. The description of trial locations.

District, Province	Soil type	Altitude (m asl)	Type of climate (Schmidt Ferguson)
Bogor, West Java	Ultisol	250	C2
Natar, Lampung	Alfisol	50	C3
Maros, South Sulawesi	Aluvia	30	D2

Table 2. Agronomic performance of sorghum germplasm.

No.	Accessions	Plant height (cm)	Stem diameter (cm)	Leaf number	Panicle length (cm)	Panicle diameter (cm)	Panicle weight (g)
1	Keris M-3	98.7	1.2	7.9	19.5	1.68	37.5
2	431	131.6	1.5	9.4	27.3	2.6	85.0
3	CK.2	104.2	1.6	10.0	28.6	2.55	61.0
4	CK.5	79.7	1.8	10.7	27.4	3.44	65.8
5	ICSV 88013	146.0	1.8	9.8	34.5	2.88	98.5
6	ICSB 70	106.6	1.4	9.6	24.9	1.97	38.5
7	Demak 4 (Mejen)	150.7	1.2	9.7	20.8	3.48	67.0
8	Somalia/Sorgum Putih	188.4	2.2	12.5	37.9	3.65	100.5
9	Rumbia (Lokal Lampung)	318.5	1.8	13.7	40.3	1.88	115.0
10	Soraya 3	120.4	1.6	9.8	18.6	3.7	66.6
11	Bioguma 1	208.2	1.6	10.4	22.8	4.0	90.5
12	Pulut 3 (NTB)	144.7	1.3	10.1	32.8	1.5	52.9
13	Samurai 2	154.5	1.5	10.5	22.0	3.9	89.2
14	Keris	58.7	0.9	7.1	20.5	1.02	19.5
15	Badik	62.3	1.1	7.0	22.3	1.4	24.9
16	867.032	82.8	0.8	6.7	18.5	1.43	22.2
17	Gadam Human	96.0	1.8	10.4	21.3	3.17	67.0
18	ICSB 11	88.2	2.2	11.2	30.5	2.67	77.0
19	K.905	74.2	1.5	8.4	28.1	1.99	44.5
20	IS 23509	166.2	1.48	9.4	28.5	1.6	49.0
21	Demak 1 (Gajah)	121.1	1.69	10.3	20.3	4.39	96.5
22	Demak 5	134.0	1.8	9.6	22.9	2.76	60.5
23	Butter Ainarup 2	126.8	1.4	8.8	30.5	1.43	75.0
24	Lokal Bima 3	230.0	1.2	9.5	39.9	1.13	59.0
25	Cantel Ketan	186.4	1.3	7.5	25.3	2.05	61.5
26	Sorgum Pulut	196.6	1.0	11.1	40.5	0.92	29.5
27	Watar Solor MEA	235.4	1.4	9.9	34.3	1.48	55.1
28	PI-150-20-A	149.0	1.6	13.8	22.3	4.06	85.0
29	Kawali	156.2	1.8	12.3	26.8	3.3	85.5
30	Pulut 5 (Bandung)	233.6	1.3	10.3	21.5	2.23	46.0

RESULTS AND DISCUSSION

Agronomic traits and amylose content

The ANOVA revealed highly significant ($P \leq 0.01$) differences among genetic materials grown for all traits observed, indicating a wide variability among them. The yield potential of the genotypes tested ranged from 19.5 to 115.0 g per panicle (Table 2). The introduced lines with relatively high yields are ICSV 88013 and 431. Among the local cultivars tested, Somalia and Rumbia are the two cultivars with the highest yield potential. Bioguma 1 and Samurai 2 are national cultivars with relatively high yield potential. Compared with the local cultivars, Bioguma 1 and Samurai 2 have better yield component traits, especially panicle diameter. The panicle diameter positively and strongly contributed to grain

yield per panicle (Wirnas *et al*., 2021; Rini *et al*., 2022). Grain size, grain number per panicle, and grain weight are vital yield component traits determining yield potential in sorghum and other cereals (Liu *et al*., 2016; Baye *et al*., 2022).

The amylose content of the tested genotypes ranged from 8.57% to 34.79% (Table 3). The highest amylose content emerged from the Somali/White Sorghum genotype, whereas the lowest came from Demak 4. In this study, lines with less than 10% amylose were considerably the waxy genotypes, as shown by the Demak 4 genotype. Based on the grouping by Rooney and Serna-Saldivar (2000) and Puspitasari *et al*. (2012), 10 genotypes classification fell under heterowaxy, namely, Keris M3, 431, CK.2, Rumbia, Pulut 3, Keris, gadam Human, Lokal Bima 3, Cantel Ketan, and 867.032, and the rest had a non-waxy type.

The average yield and amylose content of the tested genotypes based on origin are in Table 4. The highest yield potential appeared in the national cultivar group. Nevertheless, it also has a relatively high amylose content. The local cultivar group has the lowest average amylose content. However, the yield is lower than the national cultivars. Puspitasari *et al.* (2012) reported that the amylose content in mutant lines and national cultivars ranged from 22.4% to 24.8%. Sang *et al*. (2008) stated that waxy, heterowaxy, and non-waxy sorghum has an amylose content of 0%, 14%, and 23%, respectively.

The amylose content of sorghum grain depends on the dose of a recessive gene (wx). The endosperm of waxy sorghum contains three recessive waxy genes (wxwxwx), that of heterowaxy sorghum contains at least one recessive gene (WxWxwx or Wxwxwx), and the standard sorghum contains no recessive gene (WxWxWx) (Sang *et al*., 2008; Rini *et al*., 2017). Suroya *et al*. (2023) have carried out staining and molecular tests using specific markers for the waxy allele in the genotypes used in this study (Table 3). The staining results on the seeds show a dark blue color for the non-waxy genotype and reddish brown for the waxy genotype.

Table 3 presents the amylose content of sorghum genotypes based on the grouping of staining and molecular test results using specific markers for waxy alleles. Grouping the genotypes based on the type of waxy allele and amylose content revealed eight genotype groups, as in Table 5. The type of waxy allele and the staining results of the 30 genotypes tested in this study have reports from Suroya *et al*. (2023). The staining results of the non-waxy type grain showed a dark blue color and reddish brown for the waxy genotype. Twenty-four genotypes received non-waxy labels, with six genotypes classified as waxy. Among the 24 waxy lines, only 18 had the wx allele, namely wx^a and wx^c.

Based on mutations in the GBSS synthetase gene, reports revealed four alleles control the waxy trait, i.e., wx^a, wx^b, wx^c, and wx^d (Pedersen *et al*., 2005; Kawahigashi *et al*., 2013; Lu *et al*., 2013). The wx^a allele is a waxy allele commonly found in sorghum worldwide, spreading to various regions during domestication (Kawahigashi *et al*., 2013). The wx^b allele's initial discovery was in the USA and then spread to Africa, Asia, and Europe. The wx^c allele occurred in mainland Southeast Asia, Taiwan, and Tsushima Island (Japan) (Pedersen *et al*., 2007; Kawahigashi *et al*., 2013). The wx^d allele was visible in some sorghum accessions originally from China (Lu *et al*., 2013). Cho *et al*. (2015) reported no waxy genotypes containing the wx^b allele existed in the germplasm tested. Most waxy sorghum cultivars in China were either wx^a or wx^c, implying the preferential maintenance of these two mutations during domestic selection in waxy sorghum production (Lu *et al*., 2013; Maftuchah *et al*., 2022).

Until now, the waxy alleles found in Indonesian sorghum germplasm are wx^a and wx^c. Indonesian sorghum genotypes mostly carry the wx^c allele (Suroya *et al*., 2023). It was evident that six lines showed waxy traits, consisting of three genotypes with the wx^c allele, two with wx^a, and one with an unknown wx allele type, namely, the Pulut 3 genotype. This genotype is a local cultivar of Indonesia's Nusa Tenggara Barat province. A suspicion indicates that the Pulut 3 genotype has the wx^b or other wx allele.

Table 3. Amylose content of selected sorghum germplasm.

Genotype	Accessions	Allele Waxy*	Results of Staining iodine*	Amylose content (%)
G1	Keris M-3	wx^c	Non-waxy	13.67
G2	431	wx^c	Non-waxy	12.88
G3	CK.2	wx^c	Non-waxy	13.25
G4	CK.5	wx^c	Non-waxy	23.29
G5	ICSV 88013	wx^c	Non-waxy	25.17
G6	ICSB 70	wx^c	Non-waxy	32.80
G7	Demak 4 (Mejen)	wx^a	Waxy	8.57
G8	Somalia/Sorgum Putih	wx^c	Non-waxy	34.79
G9	Rumbia (Lokal Lampung)	wx^c	Waxy	13.07
G10	*Soraya 3*	-	Non-waxy	24.67
G11	Bioguma 1	-	Non-waxy	21.79
G12	Pulut 3 (NTB)	Other waxy allele	Waxy	11.88
G13	Samurai 2		Non-waxy	23.34
G14	Keris	wx^c	Non-waxy	15.95
G15	*Badik*	wx^c	Non-waxy	23.33
G16	867.032	wx^c	Non-waxy	13.57
G17	Gadam Human	wx^c	Non-waxy	15.89
G18	ICSB 11	wx^c	Non-waxy	25.24
G19	K.905	wx^c	Non-waxy	31.38
G20	IS 23509	wx^c	Non-waxy	33.26
G21	Demak 1 (Gajah)	wx^c	Non-waxy	33.33
G22	Demak 5	wx^c	Non-waxy	30.41
G23	Butter Ainarup 2	wx^c	Non-waxy	34.10
G24	Lokal Bima 3	wx^c	Waxy	19.62
G25	Cantel Ketan	wx^a	Waxy	13.56
G26	Sorgum Pulut	wx^c	Waxy	23.30
G27	Watar Solor MEA	wx^c	Non-waxy	23.31
G28	PI-150-20-A	-	Non-waxy	28.80
G29	*Kawali*	-	Non-waxy	25.37
G30	Pulut 5 (Bandung)	-	Non-waxy	25.58

Note: - = did not have a waxy allele, *Suroya *et al*. (2023).

Table 4. The mean of panicle weight and amylose content.

Groups	Panicle weight (g)	Amylose content (%)
Introduced genotypes	60.41	22.88
Local cultivars	66.29	21.34
National cultivars and IPB breeding line	71.34	24.01

Table 5. Comparison of the panicle weight and amylose content between the group of waxy and non-waxy genotypes with and without waxy allele.

Groups	Panicle weight (g)	Amylose content (%)
Waxy genotypes	64.15	*15.00*
Non-waxy with or without waxy allele	*64.20*	*24.38*
Waxy genotypes with waxy allele	*62.52*	*21.8*
Non-waxy genotypes with no wx^c allele	69.67	24.70
Non-waxy with wx^c allele	*61.95*	*24.25*
Waxy genotypes with wx^a allele	64.25	11.07
Waxy with wx^c allele	*67.83*	*18.66*

The average yield potential between the waxy and non-waxy groups was not different. The outcomes show that the waxy genotype group had moderate amylose content (Table 5). It indicates that the iodine staining method can separate sorghum genotypes with non-waxy from waxy and heterowaxy types. The waxy genotype group with the wx^a allele has a lower amylose content than the waxy group with the wx^c allele. Until now, no information is available about the type of waxy allele a genotype has with its amylose content.

Genetic parameters of amylose and agronomic traits

The heritability estimation helps get information on the proportion of genetic variability compared with environmental variability. Heritability estimation values showed that all the observed characteristics had relatively high heritability values, except for the number of leaves and amylose content (Table 6). It reveals that the observed trait variability mostly has genetic factors causing it. Rachman *et al*. (2022) reported genetic factors influenced plant height, leaf number, panicle length, diameter, and yield. The variation in amylose content and amylose/amylopectin ratio also resulted in past studies (Gerrano *et al*., 2013; Trikoesoemaningtyas *et al*., 2015).

The genetic variability in germplasm allows the selection of parents for high yield potential and moderate amylose content. The genes that control these two features transfer to the offspring of selected parents. Identifying genetic variants underlying this phenotypic variation allows plant breeders to develop genotypes with grain attributes optimized for their intended use (Boyles *et al*., 2017).

Correlation between amylose and yield traits

The results of the correlation analysis showed that yield measured as panicle weight positively correlated with other agronomic characteristics. The outcomes of this analysis also detailed that amylose content does no correlate with yield and other observed traits

(Table 7). It implies that a change in amylose content does not correlate with a change in the yield of the sorghum germplasm tested in this study. Other reports stated no correlation or a weak correlation between amylose content and yield in food crops, such as rice (Sharifi, 2018; Meena *et al*., 2020) and sorghum (Puspitasari *et al*., 2012; Boyles *et al*., 2017).

The breeding of high-quality sorghum has focused on low amylose. Information about the correlation between amylose content and yield is crucial in selection. The absence of a correlation between amylose content and yield indicates that selection for yield improvement is independent of selection for amylose content; hence, carrying out selection can use an index or independent culling to obtain high-yielding sorghum with low amylose content. Applying the independent culling selection method first requires determining the desired amylose content. In this case, developing sorghum cultivars for high yields and amylose content between 15%–20% will result in high-yielding cultivars classified as heterowaxy types.

The heterowaxy sorghum product had a good appearance and was generally comparable in quality to the non-waxy commercial (McDonough *et al*., 1998). Thus, developing sorghum cultivars must better direct at obtaining sorghum cultivars that belong to the heterowaxy type. The study findings of the heterowaxy type of sorghum are vital information pieces. Sang *et al*. (2008) reported that heterowaxy sorghum has advantages over waxy sorghum, and the physicochemical properties of its starch are not much different from the waxy type. A heterowaxy sorghum starch paste produces a soft-textured gel after the cooling process, while non-waxy sorghum starch produces a hard gel. Heterowaxy sorghum has a superior resistant starch composition than waxy. The composition of resistant starch among non-waxy, heterowaxy, and waxy sorghum is 8.4%, 23.7%, and 17.9% (Sang *et al*., 2008).

Heterowaxy sorghum also has a superior yield than waxy sorghum. The amylose content classification of heterowaxy sorghum is intermediate, which is between the amylose content of waxy and non-waxy (Sang

Table 6. Genetic parameters of agronomic traits and amylose content of the sorghum germplasm.

Characteristics	Environtment variance (Ve)	Genetic variance (Vg)	Phenotypic variance (Vp)	Broad sense heritability (h^2_{bs})	Coefficient of genetic variance (%)
Plant height	124.95	1206.81	1331.76	0.91	23.96
Stem diameter	0.01	0.04	0.04	0.81	12.63
Leaf number	9.92	0.89	0.35	0.66	9.51
Panicle length	27.04	18.16	18.40	0.99	15.76
Panicle diameter	0.52	0.01	0.01	0.91	16.69
Panicle weight	1.71	0.05	0.05	0.96	13.09
Amylose content	1.34	0.01	0.02	0.41	6.11

Table 7. Coefficient correlation between the amylose content and agronomic traits.

Characteristics	PH	SD	LN	PL	PD	PW
SD	0,099[ns]					
LN	0,488[**]	0,646[**]				
PL	0,562[**]	0,240[ns]	0,368[*]			
PD	-0,043[ns]	0,594[**]	0,531[**]	-0,338[ns]		
PW	0,424[*]	0,722[**]	0,686[**]	0,261[ns]	0,688[**]	
AM	-0,047[ns]	0,412[*]	0,240[ns]	0,086[ns]	0,206[ns]	0,208[ns]

Note: ns = nonsignificant, *, ** = significantly different.
PH= plant height, SD= stem diameter, LN= leaf number, PL= panicle length, PD= panicle diameter, PW= panicle weight.

et al., 2008). Heterowaxy sorghum is relatively stable in amylose production versus other cereals under various climatic conditions, such as stress due to high temperatures and water deficit (Yerka et al., 2016). The higher amylose content than waxy sorghum and stable amylose production can make heterowaxy sorghum yield higher. Yield is an essential concern for developing waxy sorghum in Indonesia because sorghum plant breeding aims to increase yield potential (Wirnas *et al.*, 2021).

Genotype by environment effects on amylose content

The genetic materials evaluated for the G × E effect were genotype choices from experiment 1. The locations selected for trials differed in soil type, altitude, and climate (Table 1). The results of the analysis of variance for each location showed the genotype had a highly significant effect on the amylose content. Analysis of combined variance also detailed that the genotype had a substantial influence, while location and the interaction of genetics

and location had nonsignificant effects on the sorghum's amylose content (Table 8).

Information about the G × E effect on a trait is important for a breeder to determine the selection characteristic and environment. The absence of a G × E effect on amylose content shows that selecting to improve amylose content can proceed in various environments. The absence of the G × E effect also makes it easier for breeders to select superior genotypes to release cultivars and select potential parents (Roy, 2000). Contrastingly, genotype × environment interactions indicate that selection for amylose content could continue in a cross-environment (Beta and Corke, 2001).

The mean value of amylose content of the genotypes evaluated appears in Table 9. When grouping the genetic materials evaluated in this study following the report of Puspitasari *et al.* (2012), three genotype groups resulted based on their amylose content. Group 1 (non-waxy) consisted of Keris M-3, 431, CK.5, ICSV88013, ICSB70, Somali/White Sorghum, Soraya 3, Bioguma 1, and Samurai 2, with amylose content ranging more than 20.00%.

Table 8. Analysis of the combined variance in amylose content of selected sorghum genotypes.

Source of variation	df	MS	SS	*P* value
Locations (L)	2	353.55	176.77*	0.0190
Replications (Locations)	6	128.71	21.45[tn]	0.6844
Genotypes (G)	12	3,367.26	280.60**	0.0000
G*L	24	422.99	17.62[tn]	0.9540
Error	72	2,351.03	32.65	
Total	116	6,623.55		

Note: * = significant at α 5%; ** = significant at α 1%.

Table 9. Amylose content of selected sorghum genotypes in three locations.

No	Accessions	Bogor (%)	Natar (%)	Maros (%)	General mean
1	Keris M-3	20.77	23.2	18.42	20.81[a]
2	431	21.50	22.3	18.22	20.68[a]
3	CK.2	20.99	21.3	16.02	19.42[ab]
4	CK.5	24.94	22.2	15.74	20.95[a]
5	ICSV 88013	23.23	20.1	21.59	21.65[a]
6	ICSB 70	27.83	24.7	25.21	25.92[a]
7	Demak 4 (Mejen)	9.38	13.0	7.370	9.90[c]
8	Somalia/Sorgum Putih	28.81	17.0	22.99	22.93[a]
9	Rumbia (Lokal Lampung)	10.74	6.56	5.90	7.73[c]
10	*Soraya 3*	24.67	22.2	17.03	21.31[a]
11	Bioguma 1	21.79	22.2	17.40	20.45[a]
12	Pulut 3 (NTB)	11.88	8.9	10.80	10.51[bc]
13	*Samurai 2*	23.35	22.6	18.00	21.32[a]
	HSD				9.46
	Mean	20.76[a]	18.94[ab]	16.52[b]	

Note: Numbers in the same column and followed by the same letter are not significantly different in the Tukey 5%.

Pulut 3 and CK.2 belonged to Group 2 (heterowaxy), with an amylose content of 10.51% and 19.40%, respectively, and Group 3 (waxy type) included Demak 4 and Rumbia (Table 9). Demak 4 and Rumbia are two genotypes that show consistency as waxy genotypes because both are positive for iodine staining, and each has the wx^a allele for Demak 4 and wx^c for Rumbia.

When focusing on the data for each replication and each location grown in three areas of IPB lines and national cultivars (Soraya 3, Bioguma 3, and Samurai 2), it can be apparent that almost all genotypes have a lower variation (Figure 1). National cultivars and breeding lines are genotypes that attained selection; hence, their genetic constitution is a homogeneous homozygous population. The variability of the homozygous homogeneous populations resulted from environmental factors' variability (Roy, 2000; Acquaah, 2012). On the other hand, local cultivars show higher variability because local cultivars are homozygous heterogeneous populations. In addition, local cultivars have high adaptability to specific agroecological conditions.

CONCLUSIONS

The results showed genetic variations in amylose content and yield traits of sorghum germplasm, with a high heritability for yield traits and a medium heritability for amylose content. No correlation existed between amylose content and yield. Genotypes had highly significant effects, while location and the interaction of genotypes and location had nonsignificant influences on the amylose content of sorghum. Pulut 3 and CK.2 are genotypes with consistent classification as heterowaxy, with Demak 4 classified as waxy sorghum when grown in three different environments.
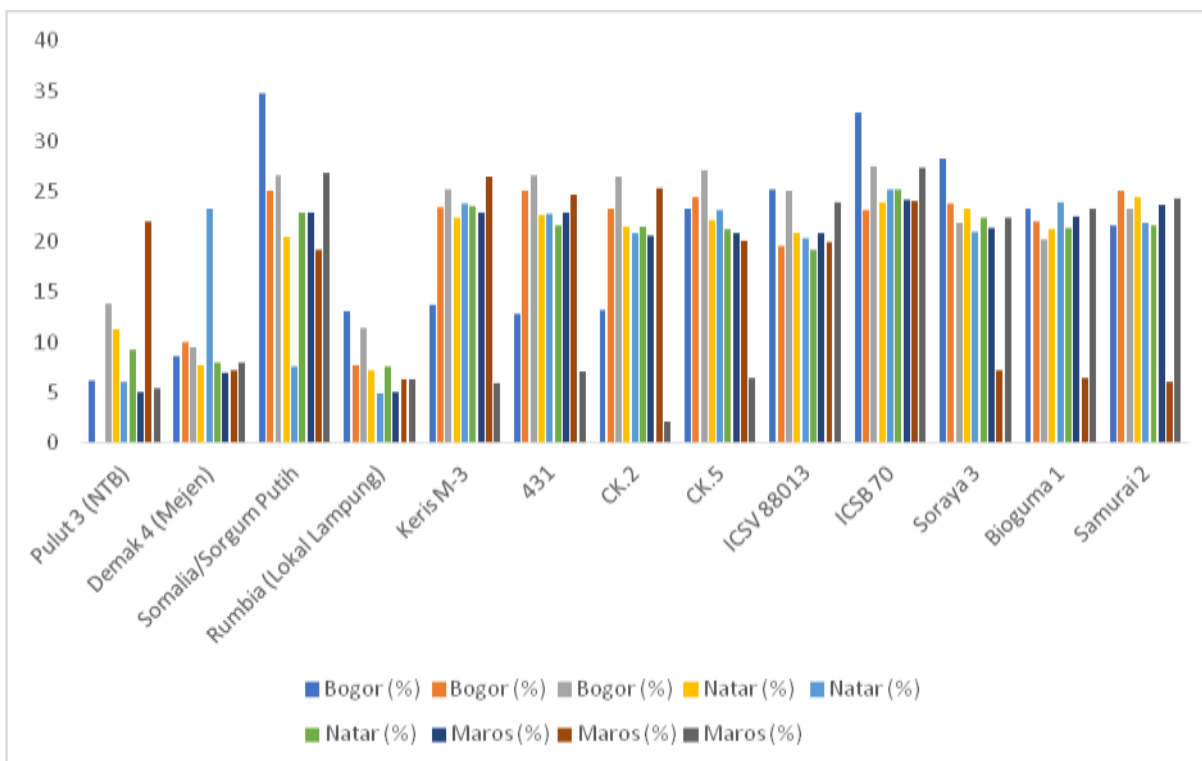
Figure 1. Amylose content of selected sorghum genotypes in three locations.

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