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Estimation of genetic parameters and selection of sorghum [*Sorghum bicolor* (L.) Moench] RILS F5 derived from single seed descent

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Key words: Single seed descent, Augmented design, Heritability, selection differential.

Abstract

The purpose of this study was to obtain information about genetic parameters of agronomic characters of sorghum lines developed by Single Seed Descent as information for yield improvement through selection. The research was conducted from July to October 2014 in Bogor, West Java, Indonesia with an altitude of ± 240 m above sea level and a temperature of 27°C. The genetic materials used consisted of 201 RILs F5, and Numbu, Samurai-1, and Samurai-2 (national varieties), and a mutant B69 as check varieties. The experimental design used was augmented design. The results showed that the RILs F5 significantly different in the characters of seed filling period, plant height, leaf number, panicle length, circumference panicle, panicle weight, and grain weight panicle^{-1}. High broad sense heritability values and broad genetic diversity were observed in the character of the seed filling period, plant height, leaf number, panicle length, circumference panicle, panicle weight and grain weight panicle^{-1}. There were RILs F5 which have higher yield than the two parents and are uniform with lower within line variance. Selection was conducted based on grain weight panicle^{-1} increased 35.3% yield, but at the same time increased plant height by 5%. Simultaneous selection by grain weight panicle^{-1} and plant height increased yield by 21% and reduced plant height by -6.9%. This gives the opportunity to obtain shorter high yielding varieties.

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Introduction

Sorghum is an important food crop in the world after rice, wheat, maize, and barley (Reddy *et al.*, 2012). Sorghum is a multifunctional crop that can be used as a source of food, feed and biofuel (Dicko *et al.*, 2006). Besides, it has many benefit, sorghum also has wide adaptability because it needs fewer inputs compared with other crops. Sorghum is a tolerant crop to drought conditions (Biba, 2011).

As a food commodity sorghum has not been given priority to be developed in Indonesia. This is because the comparative value and competitive advantages of sorghum are low (Sirappa, 2003). The productivity of sorghum in Indonesia in 2011 only reached 2.1 tons ha^{-1} (Directorate General of Food Crops, 2013). By growing high yielding varieties, farmers can harvest sorghum up to 5 tons ha^{-1} (Kelley *et al.*, 1992). Therefore, it is necessary improved varieties of sorghum for higher yield.

Increasing genetic variability of plant populations is the first step in a plant breeding program (Phoelman, 2006). Wide genetic variability is the basis for the success of selection to produce superior varieties. Genetic variability can be generated through introduction, mutation, or hibridizaition (Aquaah, 2007). Efforts to increase the genetic variablity in sorghum through hybridization in Indonesia is still limited. The existing high yielding national varieties of sorghum were developed through introduction and mutation techniques. The number of the national sorghum varieties are limited so the effort to develop sorghum varieties is still need to be done.

The Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University has conducted sorghum breeding program through hybridization since 2007. A cross of B69 × Numbu was conducted and through single seed descent (SSD) method has resulted in 201 F5 recombinant inbred lines (RILs). Recombinant inbred lines developed through SSD have high variablity as the F2 generation with uniformity within the lines (Kong *et al.*, 2013; Phuong *et al.*, 2013; Ibrahim 2015).

The yield components are controlled by many genes whose expression is strongly influenced by the environment (Miladivonic *et al.*, 2011). The variability of the yield character in F5 RILs resulted from SSD is caused by additive gene actions because each line is derived from a self-pollinated plant. According to Mangoendidjojo (2003), a selection in the next generation of the lines obtained from SSD method would be more effective as additive genes contribute to the genetic variability has become larger and the resulting lines are increasingly homozygous.

Estimation of genetic parameters is aimed to obtain basic information for improvement of characters through selection (Wardina and Pranowo, 2011). Estimation of genetic parameters for sorghum produced by SSD method has not been widely studied further. It is therefore necessary to conduct genetic studies to support the selection of superior varieties of sorghum. The purpose of this study was to obtain information on the genetic parameters in a population of RILs developed through SSD and to conduct selection for high yielding lines based on the information.

Materials and methods

Place and time of study

The study was conducted from July to October 2014 in Bogor, West Java, Indonesia at the altitude of 240 m above sea level and the average maximum temperature of 27°C and minimum of 25°C.

Genetic materials

The genetic materials used in this study were 201 RILs of sorghum (B69 x Numbu) developed throgh SSD method and 3 national varieties, namely Numbu, Samurai-1, Samurai-2, and a mutant line (B69) as check varieties.

Experimental design

The study was conducted in an augmented design with genotypes as treatments. Genotype consists of 201 RILs which were not replicated and the control varieties were replicated 6 times to estimate the error variance.

Working proceedures

Tillage was done one week before planting. Each RILs and the control varieties planted in a single row. Spacing whitin a row was 10 cm, while the distance between rows was 75 cm. The appllication of urea fertilizer was conducted twice, at the time of planting 50 kg ha^{-1} in conjunction with SP-36 and KCl respectively 100 kg ha^{-1}. The second urea application was performed at age 4 weeks after planting at a dose of 100 kg ha^{-1}. Harvesting was done when 80% of the crop was ripe characterized by the appearance of black layer on the seed. Observations were made on the characters of harvest time (days), days to flowering (days), grain filling period (day), rate of grain filling (gr day^{-1}), plant height (cm), stem diameter (mm), number of leaves, panicle length (cm), panicle circumference (cm), weight of panicle (g), and grain weight panicle^{-1}(g).

Data analysis

Data were analyzed based analysis for model augmented (Sharma,1988). Analysis of data was conducted using Minitab software 16.2.1, and SAS version 9.0. Estimation of the value of variability was based on the expected mean squares (Syukur *et al.*, 2010); σ^2_e = EMS/ r, σ_{2g} = (MSG-EMS) / r, $\sigma^2_p = \sigma^2_g + \sigma^2_e$. the EMS description = error mean squares; GMS = genoype mean squares; r = replication; $\sigma^2 e$ = environments variability, σ^2_p = phenotypes variability, σ^2_g = genetic variability.

Estimation of broad sense heritability was based on a ration between a genetic variability (σ^2_g) to the phenotype variability (σ^2_p) with a criteria: high $h^2_{bs} > 0.5$, was $h^2_{bs} \leq 0.5 \leq 0.2$; and low $h^2_{bs} < 0.2$. Heritability values calculated according to Roy, 2000) by the equation of $h^2_{bs} = \left(\frac{\sigma^2_g}{\sigma^2_p}\right) \times 100$.

The value genetic variability was determined based on genetic variation (σ^2_g) and standard deviation ($\sigma_{\sigma^2 g}$) according to the following formula: $\sigma_{\sigma^2 g} = \sqrt{2} / r2 \, [(MSg^2 / DBG + 2) + (MSe^2 / dbe + 2)$, EME description = error means square; KTG = squared middle strain; r = replication; db= degrees of freedom form line; dbe = degrees of freedom error. If $\sigma^2_g > 2\sigma_{\sigma g}$: broad genetic diversity, whereas $\sigma^2_g < 2\sigma_{\sigma g}$: narrow genetic diversity (Pinaria *et al.*, 1995).

Progress predictive value selection can be formulated as follows $G = I.\sigma_p.h^2_{bs}$; I = intensity of selection at a rate of 15% in the amount of 1.6, standard deviation σ_p = phenotype, h^2_{bs} = broad sense heritability (Falconer and Mackay, 1996). Variety in the lines and between the lines RIL F5 suspected of diverse phenotypes in RILs F5.

Variety is calculated based on the phenotype observed Gomez and Gomez (2007), namely $\sigma^2 = \sum_{n-1}^{i} {(xi - x)^2}/{n - 1}$. Description: σ^2 = the value of variability within line or between strains, xi = the average of each individual or each line, x = the line means, n = number of observations.

Selection of high yielding lines was carried out by one character (character grain weight panicle^{-1}) with a selection intensity of 15% and based on two characters at once (grain weight penicle^{-1}and plant height). Then proceed to calculate the selection differential by the equation: $S = (X_i - X_o/X_o)$ x100%; S = differential selection, (X_i) = average of selected populations, (X_o) = average of initial population (Falconer and Mackay, 1996).

Results and discussion

Sorghum lines evaluated in this study were Recombinant inbred Lines (RILs) F5 developed by Single Seed Descent (SSD) from crosses between B69 × Numbu. Each RILs F5 grown into one line. The RILs were the result a single plant of the previous generation.

A t-test showed that the national variety Numbu and mutant line B69 were different in plant height, leaf number and grain weight panicle^{-1}. The mean performance of the agronomic characters of Numbu were generally better than B69 (Table 1). Differences in the performance of agronomic characters in the two parents was attributed to difference in adaptability of B69 and Numbu. According ICERI (2014), it is known that Numbu is one of the national varieties that has broad adaptation with the potential yield of 4-5 tones ha^{-1}.

Table 1. Variance analysis (ANOVA) based on augmented design (Sharma, 1988).

Source of Variation	Df	SS	MS	EMS
Block	b-1	Jkb	Ktb	
Genotype (p)	(g+c)-1	JKp	KTp	
Line (g)	g-1	JKg	KTg	$\sigma_e^2 + \sigma_g^2$
Control (c)	c-1	JKc	KTc	$\sigma_e^2 + r\sigma_c^2$
g vs c	1	JK(g vs c)	KT (g vs c)	
Error	(c-1)(b-1)	Jke	Kte	σ_e^2
Total	(bc+g)-1	JKT		

Table 2 shows that the mean population RILs F5 exceed the mean of the two parents for the characters of days to harvest, grain filling period, stem diameter, leaf number and panicle circumference. This means that there are lines developed from potential segregants that has the performance higher than both parents. For the character of days to flowering, grain filling rate, plant height, panicle length, panicle weight and grain weight panicle^{-1} the mean values of RILs were between the mean value of the two parents. The range of the value in the RILs population was wide, which indicates that there are some RILs F5 better than the mean value of the two parents.

Table 2. The range and mean of agronomic characters of RILs F5, Numbu, and B69.

Character	Numbu	B69	t-value	F5 mean	F5 Range
Days to flowering (days)	65.0± 5.7	64.0±3.0	1.2	64.3	55.0 - 76.0
Harvest time (days)	103.0± 1.2	103.0±1.2	0.0	104.0	88.0 - 117.0
Grain filling period (days)	36.3± 4.6	39.3±2.7	1.4	39.5	27.0 - 60.0
Rate of grain filling (g day^{-1})	1.5± 0.7	1.1±0.1	1.5	1.4	0.4 - 3.1
Stem diameter (mm)	13.5± 2.1	12.8±1.1	0.7	13.8	8.5 - 18.4
Plant height (cm)	224.0±15.5	196.4±5.5	4.1*	217.0	157.4 - 275.8
Number of leaves	10.1± 0.6	9.2±0.7	2.5*	10.3	7.0 - 13.0
Panicle length (cm)	17.3± 1.4	18.6±1.6	1.5	18.5	11.8 - 26.2
Panicle circumference (cm)	14.6± 2.1	13.4±0.8	1.4	14.9	9.6 - 20.1
Weight of panicle (g)	74.3±22.2	58.4±5.3	1.7	62.4	10.6 - 113.8
Grain weight panicle^{-1}(g)	56.2± 6.9	44.3±2.5	3.9*	55.2	21.2 - 101.2

Note: * = significantly different by t test at level α = 5%.

The results of the variance analysis showed that the lines were significantly different for the characters of seed filling period, plant height, leaf number, panicle length, panicles circumference, panicle weight, and grain weight panicle^{-1} (Table 3). Advanced generation lines resulting from crosses between significantly different parents show the high variability of agronomic characters and yield. This is consistent with research Arunkumar *et al.*, (2004); Puspitasari *et al.*, (2012); Sami *et al.*, (2013a); Kamatar *et al.*, (2015).

Table 3.Variance analysis on agronomic traits of sorghum RILs population.

Character	Source of Variation					
	Genotype		Line		Control	
	MS	F-Value	MS	F-Value	MS	F-Value
Days to flowering (days)	23.3	1.5	23.1	1.5	23.3	1.5
Harvest time (days)	20.5	1.2	20.5	1.2	23.4	1.4
Grain filling period (days)	41.7	2.3*	40.8	2.3*	60.4	3.3*
Rate of grain filling (g day^{-1})	0.2	1.0	0.2	0.9	0.4	1.8
Stem diameter (mm)	4.1	1.7	3.8	1.6	16.9	7.2**
Plant height (cm)	532.7	2.6*	514.7	0.0*	1603.1	7.9**
Number of leaves	1.4	2.4*	1.2	2.1*	12.4	21.2**
Panicle length (cm)	9.0	3.3*	6.9	2.5*	92.9	33.7**
Panicle circumference (cm)	3.6	2.2*	3.1	1.9*	11.2	6.8**
Weight of panicle (g)	381.4	2.7*	378.7	2.7*	630.6	4.5*
Grain weight panicle^{-1} (g)	267.1	5.4**	257.3	5.2**	370.3	7.5**

Note: * = significant at the 5% level. ** = Significant at the 1% level.

The expected variability observed in the population tested is caused by the genetic factors. Selection will be efficient when conducted in populations that have large genetic variability (Phoelman and Sleeper, 2006). Therefore, it is important to estimate the variance components and heritability value of RILs F5 in order to know the genetic variability contribution to the variability of phenotypes.

The advanced generation RILs F5 resulted from SSD can retain the genetic variability. This is evidenced by the majority of agronomic characters in RILs F5 have a broad genetic diversity and high heritability (Table 4). According to Roy (2000), the advanced generation SSD can retain genetic variablity as the earlier generations and the advanced lines have high variability among lines with low variability within lines.

The characters that have broad genetic variability and high heritability estimates in the advanced generation of SSD indicate that the characters are influenced by additives gene action. According to Mangoendidjojo (2003), in the population of SSD, selection is delayed until the advanced generation, this resulted in the fixation of additive genes. This makes the value of h^2_{bs} as a good estimate of the h^2_{ns}. According to Suprapto and Kairudin (2007), a high heritability is useful in the selection if the genetic variance is mainly consists of additive genes because the additive effect of each allele will be inherited from the parents to the progenies.

In general, the observed character has a predictive value of a positive selection gain (Table 4). This indicates an improvement in mean values in the next generation. According to Falconer and Mackay (1996), the hight value of selection gain reflects the improvement of the character achieved when selection is conducted. The higher the value of expected selection gain of a character the more effective the selection. The estimation of selection gain for plant height and weight of grain panicle^{-1} showed the highest estimated value of selection gain (Table 4).

Table 4. Genetic parameters for agronomic characters of Sorghum RILs population.

Character	σ^2_e	σ^2_g	σ^2_p	H_{bs}	$2 (\sigma_{\sigma^2 g})$	ΔG
Days to flowering (days)	2.6	1.2	3.8	31.0^{S}	1.9^{S}	1.0
Harvest time (days)	2.8	0.6	3.4	16.0^{R}	2.1^{S}	0.5
Grain filling period (days)	3.0	3.8	6.8	56.0^{T}	2.5^{L}	2.3
Rate of grain filling (g day^{-1})	0.0	0.0	0.0	0.0^{R}	0.0^{S}	0.0
Stem diameter (mm)	0.4	0.3	0.6	39.0^{S}	0.3^{S}	0.5
Plant height (cm)	33.7	55.1	85.8	60.0^{T}	28.7^{L}	8.9
Number of leaves	0.1	0.1	0.2	52.0^{T}	0.1^{L}	0.4
Panicle length (cm)	0.5	0.7	1.1	60.0^{T}	0.4^{L}	1.0
Panicle circumference (cm)	0.3	0.3	0.6	50.0^{T}	0.2^{L}	0.6
Weight of panicle (g)	23.5	39.7	63.1	63.0^{T}	20.4^{L}	8.0
Grain weight panicle^{-1} (g)	8.2	34.7	42.9	81.0^{T}	10.2^{L}	8.5

Note: σ^2_g = genetic variance; σ^2_p = phenotypic variance; σ^2_e = environtment variance; h^2_{bs} = Broad sense heritability; $\sigma_{\sigma^2 g}$= standard deviation of genetic diversity.R=low, S=Medium, L=High. $\sigma_{\sigma^2 g}$:S=narrow, L=wide, G=Genetic advance value.

Quantitative character is controlled by polygenes so that the selection will be effective if it is done in advanced generation when the homozygous is already high (Aquaah, 2007). According to Sulistiowati (2015), the early generations of sorghum resulted from a crossing B69 × Numbu shows that all agronomic characters are controlled by additive gene action. Therefore, the selection is done in advanced-generation with SSD method. In the advanced generation fixation of additive genes becomes higher, which causes variations whithin lines to be low (Allard, 1960). The characters observed in the RILs have within line genetic variability lower than between lines variability (Table 5). This indicates that each RIL is uniform within line. When the lines have lower variability within lines compared to between lines, then there is a posibility to obtain potential lines (Aquaah, 2007).

Table 5. Mean, variance within lines, and variance between lines of sorghum RILs population.

Character	Mean	Variance within lines	Variance between lines
Rate of grain filling (g day^{-1})	1.4 ± 0.5	0.1	0.2
Plant height (cm)	217.1 ± 23.0	272.9	530.9
Stem diameter (mm)	13.8 ± 2.1	2.8	4.2
Number of leaves	10.3 ± 1.2	0.8	1.3
Panicle length (cm)	18.5 ± 2.7	2.3	7.6
Panicle circumference (cm)	14.9 ± 1.9	1.9	3.5
Weight of panicle (g)	62.4 ± 20.5	149.0	421.9
Grain weight panicle^{-1} (g)	55.2 ± 17.2	96.6	296.4

The development of pure lines in this study is aimed at high yielding. Direct selection for yield can results in large improvement if the character of yield has a high heritability (Sarwar *et al.*, 2004, Wirnas *et al.*, 2006). The grain weight panicle^{-1} can be effectively used as selection character because it has a broad genetic variability. The value of expected selection gain and high heritability values of weight of grain panicle^{-1} are relatively high compared to other yield characters. Similar results were reported by Chavan *et al.*, (2010); Rani and Umakanth, (2012); Sami *et al.*, (2013b).

Selection of a single character based on the weight of grain panicle^{-1} with a selection intensity of 15%, low within line variability, and mean value higher than the two parents, resulted in 30 selected lines. This selection also can increase grain weight panicle^{-1} 35.3%, weight of panicle 37.7%, panicle circumference 12%, panicle length 9.2%, number of leaves 1%, plant height 5%, stem diameter 12.3%, Rate of grain filling 21.4%, Grain filling period 8.6%, as well as 0.6% and -4.4% for days to harvesting. and flowering, respectively (Table 6). This means that a selection based on the weight of grain panicle^{-1} can also improved the agronomic characters in addition to yield.

Table 6.Differential selection based on the grain weight panicle^{-1}.

Character	Average initial population	Average population selected	Differential selection (%)
Days to flowering (days)	64.3	61.5	-4.4
Harvest time (days)	103.8	104.4	0.6
Grain filling period (days)	39.5	42.9	8.6
Rate of grain filling (g day^{-1})	1.4	1.7	21.4
Stem diameter (mm)	13.8	15.5	12.3
Plant height (cm)	217.1	227.9	5.0
Number of leaves	10.3	10.4	1.0
Panicle length (cm)	18.5	20.2	9.2
Panicle circumference (cm)	14.9	16.7	12.1
Weight of panicle (g)	62.4	85.9	37.7
Grain weight panicle^{-1} (g)	55.2	74.7	35.3

Selection based on grain weight panicle^{-1} result in increasing plant height of the selected lines. Increase in plant height is not required in the development of sorghum as a food resources. Plants that can lead to higher risks of increasing stem felling and harvesting as well as the difficulty in maintenance (Sungkono, 2009). Therefore, direct selection is based on two character, namely grain weight panicle^{-1}and plant height to cope with effective risk stems fall need to be done. Table 7 shows that selection based on plant height and grain weight panicle^{-1} produce 20 selected. The results of this selection led to an increase in differential selection grain weight panicle^{-1} 21.0% and reduce plant height -6.9% (Table 7). This means that the selection is based on the weight of grain panicle^{-1} and the plant height is able to obtain an increase in grain weight panicle^{-1} and lower plant height lower than Numbu.

Table 7. Differential selection based on the grain weight panicle^{-1} and plant height.

Character	Average initial population	Average population selected	Differential selection (%)
Days to flowering (days)	64.3	62.1	-3.4
Harvest time (days)	103.8	105.4	1.5
Grain filling period (days)	39.5	43.3	9.6
Rate of grain filling (g day^{-1})	1.4	1.5	7.1
Stem diameter (mm)	13.8	14.3	3.6
Plant height (cm)	217.1	202.2	-6.9
Number of leaves	10.3	9.6	-6.8
Panicle length (cm)	18.5	20.8	12.4
Panicle circumference (cm)	14.9	15.7	5.4
Weight of panicle (g)	62.4	72.6	16.3
Grain weight panicle^{-1} (g)	55.2	66.8	21.0

Strains are selected based on two characters at once expected to have the high fixation additive gene so as not to cause further segregation in the lines mainly on plant height and grain weight panicle^{-1}. The selected lines could be evaluated in preliminary yield.

Conclusion

RILs F5 sorghum shows high variation due to genetic factor. Most of the traits observed shows a high heritability. It is estimated that additive genetic factors affected variation between the lines observed because population was generated through SSD. Direct Selection is based on plant height and grain weight panicle^{-1} simultanously is more effective than the selection one based on grain weight panicle^{-1} only to improve yield potential and plant height.

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