Diversity and inheritance in cowpea (*Vigna unguiculata*) on protein and yield components characters

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Abstract. *Purnamasari I, Sobir, Syukur M. 2019. Diversity and inheritance in cowpea (Vigna unguiculata) on protein and yield components characters. Biodiversitas 20: 1294-1298.* Diallel was a method that can be used to see inheritance and diversity character of plants. The objective of this study has evaluated the inheritance and diversity of the yield component characters and seed protein content. This study used genetic material from five homozygous cowpeas (*Vigna unguiculata* (L.) Walp) genotypes (KM1, KM2, KM4, KM5, and TG2) and 20 F1. Genotypes were planted using a randomized complete block design with three replications. The character observed were the yield components (weight of 100 seed, number of seeds per pod, number of pods per bunch, number of pods per plant and yield) and seeds protein content. Characters that were influenced by general combining ability (GCA) consist of weight of 100 seeds, number of pods per bunch, number of seeds per pod and seed protein content. The variance of GCA has a greater value than the variance of specific combining ability (SCA). Narrow sense and broad sense heritability values were high category consist of weight 100 seed, number seeds per pod, number pods per bunch and seed protein content. That characters were inherited additively. Characters were influenced by additive gene action and have high diversity make it possible to select in F2 generation using the pedigree selection method.

Keywords: Additive, diallel, GCA, heritability, SCA

INTRODUCTION

Legumes are one food source that has many useful ingredients needed by humans. The most important ingredient in legumes is protein. Protein in legumes is a type of vegetable protein, a source of protein that is contained in plants. Proteins are useful as activators of the immune system for protection against foreign organisms and several other important functions (Fürst 2009). Soybean is a source of vegetable protein that is widely used as a basic ingredient in food products. The protein contained in soybeans is 38 grams of protein per 100 soybean seeds. Soybeans can produce optimally if planted in areas with irradiation of more than 12 hours namely in subtropical countries, so other commodities are needed for soybean substitution to meet vegetable protein needs.

One type of legumes that has the same physical shape as soybeans are cowpea. Cowpea (*Vigna unguiculata* (L.) Walp) is vegetable beans that are usually harvested before and after physiologically mature. Cowpea pods are longer than soybeans, which causes a very significant difference in the number of seeds per pod. Cowpea contains 22 grams of protein per 100 seeds, so that it has the potential to be increased to be equal or close to the value of soy protein content. Character repairing in plants requires the desired diversity of characters, making it easier in the selection process. The higher the diversity of characters who want to be improved, the selection process will be better and can increase the response to selection value. Increasing diversity in plants can use mutations (Molina 2017; Nair 2014), molecular (Buu and MyMy 2003; Boukar et al. 2016), and crosses (Mbusa et al. 2018; Jean Baptiste 2011).

Crossing is the method most often used to increase diversity and improve quality character of plant. The diallel mating design is most commonly used by plant breeders. The design allows a combination of crosses between all the genotype chosen by considering different characters. In diallel crossing can be known the value of combining ability which gives information to breeders about the actions of genes involved in inheritance of a character and diversity of characters (Hayman 1954; Griffing 1956; Aghao et al. 2010; Daryanto et al. 2010; Syukur et al. 2010; Aisya et al. 2016; Ganefianti et al. 2018). Based on this description, this study was conducted to obtain information about inheritance in cowpea using diallel crossing with a griffing approach to the character of the yield components and protein content of the seeds.

MATERIALS AND METHODS

Field experiments were conducted at Ponci, Sulawesi Selatan, Indonesia. The line used in this study consist of five homozygous parents (KM1, KM2, KM4, KM5, and TG2) and 20 F1 hybrids. The hybrids f1 and reciprocals were made by the full Diallel cross method. The parents and F1 were evaluated in a randomized complete block design (RCBD) with three replicates. Each block was

divided into 25 plots each measuring 0.8 m². Seedlings were grown at a spacing of 40cm x 10 cm inter-row. Parents and F1 were randomly assigned to each block and labeled accordingly. Fertilization was done a week after planting (7 days), fertilizing using Urea 2.5 kg ha⁻¹, TSP 45 kg ha⁻¹, and KCl 45 kg ha⁻¹. In the flowering stage, the experimental plot was sprayed with lambda-cyhalothrin 2 ml l-1 insecticide once a week to suppress fruit borers (Indiana 2007) and sprayed Nissuron 50 EC to control aphid fleas.

Data collect

The data collected on the yield characters consisted of weight of 100 seeds were count 100 seed dried per plot, number of seeds per pod were counted 1 pod per bunch , number of pods per bunch were count number of pod per bunch in sample plants, number of pods per plant were count number of pods per plant in sample plants, yield per plant were count all of the dried seeds in sample plants and seed protein content were analysis used Kjeldahl analysis. each yield character collected 10 sample per plot totally 75 plot consist parent, f1 and f1r while seed protein content using 45 samples consist of parents and f1. Data of character results were analyzed using DiallelGriffing Method 1 while seed protein content was analyzed using Diallel Griffing Method 2.

Data analyses

Analysis variance (ANOVA) and genetic analysis weight of 100 seed, number of seed per pod, number of pods per bunch, number of pods per plant, yield per plant, and seed protein content were analyzed using AGD-R v4.0. Analysis variance using probability 1% for highly significant and 5% for significant. DiallelGriffing's Method 1 (Parents, F1, Reciprocal F1 hybrid) and model 2 (random model) were used to estimate the general combining ability (GCA), specific combining ability (SCA) and reciprocals effect for yield component (Sing and Chaudhary 1979). DiallelGriffing's Method 2 (Parents, F1, excluding reciprocal F1 crosses) and model 2 (random model) were used to estimate the general combining ability (GCA) and specific combining ability (SCA) for seed protein content.

$MS_E' = \frac{MS_E}{r}$, with r = block

$\sigma^2_E = MS_E'$

$\sigma^2_g = \frac{1}{2p}\left[MS_g - \frac{MS'_E + p(p-1)MS_s}{p^2 - p + 1}\right]$

$\sigma^2_s = \frac{p^2}{2(p^2 - p + 1)}[MS_s - MS'_E]$

$\sigma^2_r = \frac{1}{2}[MS_r - MS'_E]$

Additive variance and dominant variance were obtained from general combining ability (GCA) and specific combining ability (SCA) if all of diallel assumptions were covered (Griffing 1956);

$\sigma^2_A = 2\sigma^2_g$

$\sigma^2_s = \sigma^2_D$

General combining ability (GCA), specific combining ability (SCA), and variance of protein content assumptions were using diallel method 2.

Table 1. Anova combining ability Diallel Method 1 with references to Sing and Chaudhary (1979).

Source of variation	Degree of freedom	Mean square	Expected mean square
Replication	r-1	MS_R	
Genotype	v-1	MS_G	$\sigma^2_E + r\,\sigma^2_G$
GCA	(p-1)	MS_g	$\sigma^2_E + \frac{2(p^2 - p + 1)}{p^2}\sigma^2_s + 2p\sigma^2_g$
SCA	(p-1)	MS_s	$\sigma^2_E + \frac{2(p^2 - p + 1)}{p^2}\sigma^2_s$
Reciprocal	$\frac{1}{2}p\,(p-1)$	MS_r	$\sigma^2_E + 2\sigma^2_r$
Error	(r-1)(v-1)	MS_E'	σ^2_E

Table 2. Anova combining ability Diallel Method 2 with references to Sing and Chaudhary (1979)

Source of variation	Degree of freedom	Mean square	Expected mean square
Replication	r-1	MS_R	
Genotype	v-1	MS_G	$\sigma^2_E + r\,\sigma^2_G$
GCA	(p-1)	MS_g	$\sigma^2_E + \sigma^2_s + (p+2)p\sigma^2_g$
SCA	(p-1)	MS_s	$\sigma 2E + \sigma 2s$
Error	(r-1)(v-1)	MS_E'	σ^2_E

$MS_E' = \frac{MS_E}{rs}$, with r = block dan s = sample

$\sigma^2_E = MS_E'$

$\sigma^2_g = \frac{1}{(p+2)}[MS_g - MS_s]$

$\sigma^2_s = [MS_s - MS'_E]$

Additive variance and dominant variance were obtained from general combining ability (GCA) and specific combining ability (SCA) if all of diallel assumptions were covered (Griffing 1956);

$\sigma^2_A = 2\sigma^2_g$

$\sigma^2_s = \sigma^2_D$

Broad-sense heritability (h² ₍bs₎) dan narrow-sense heritability (h² ₍ns₎) were obtained from Diallel analysis method 1 and method 2 (Sing dan Chaudhary 1979).

$h^2bs = \frac{2\sigma^2GCA + \sigma^2SCA}{2\sigma^2GCA + \sigma^2SCA + \sigma^2E}$

$h^2ns = \frac{2\sigma^2GCA}{2\sigma^2GCA + \sigma^2SCA + \sigma^2E}$

RESULT AND DISCUSSION

Combining ability

Yield component

Yield component consists of 100 seeds weight, number of seeds per pod, number of pods per bunch, number of pods per plant, and yield per plant (Table 3). In the analysis of variance, it was found that the general combining ability (GCA) the weight of 100 seeds was significant at 0.01 level, whereas in peanuts (Neya et al. 2017) the results showed that the weight of 100 seeds has been significant at specific combining ability (SCA). The number of seeds per pod was significant at the 0.01 level different from the results of the study by Dahosa and Alika (2013), the number of seeds per pod has been significant value, in

general, combining ability (GCA) and specific combining ability (SCA) while in peanut (Jogloy *et al.* 2005), the yield character pods, seed yield, and weight of 100 seeds have been significant in general combining ability (GCA). The number of pods per bunch was significantly 0.05 in the general combining ability (GCA) while the character of the number of pods per plant and yield per plant was not significant in all components of variance, whereas the results obtained by Patel et al. (2010) showed pods per plant was significant in specific combining ability (SCA) and the results obtained Alam (2013) was significant on SCA and GCA in peanuts.

Protein content

Protein content was significant at the 0.01 level on general combining ability (GCA) while the specific combining ability (SCA) was not significant at the level of 0.05 and 0.01, which is different from the results of studies on wheat and Indian beans (*Dolichous lablab* L) (Patil et al. 2013; Al-Naggar 2015) protein content in wheat have been significant at 0.01 level on general combining ability (GCA) and specific combining ability (SCA).

Variance

Yield component

The yield components included weigh of 100 seeds, number of seeds per pod, number of pods per bunch, number of pods per plant, and yield per plant had a higher GCA value than the SCA value (Table 5). The value of heritability in a broad sense and narrow sense can be obtained from variance values. The heritability values indicate the broad sense heritability and narrow sense heritability on all components of the yield in the high category except yield per plant (Syukur et al. 2010) with a broad sense heritability and narrow sense heritability of weight 100 seed each 0.98 and 0.98, number of seeds per pod 0.99 and 0.98, number of pods per bunch 0.91 and 0.90, number of pods per plant 0.95 and 0.85, yield per plant 0.89 and 0.45, according the results of narrow sense heritability values in wheat (Al-Naggar 2015). Heritability ratio was used to determine the additives variance on genetic variance, heritability ratio on the weight of 100 seeds, number of seeds per pod, number of pods per bunch, number of pods per plant and yield per plant each 100%, 99%, 99%, 90%, and 51%.

Protein content

Protein content has a variance of GCA values greater than the variance of SCA. The broad sense heritability and narrow sense heritability of the protein content of the seeds is included in the high category with each value of 0.918 and 0.745 (Syukut et al. 2010), according to the results of narrow sense heritability values in wheat (Al-Naggar 2015) narrow sense heritability of protein content was in the low category in all nitrogen treatments but in the broad sense heritability of protein content was in the high category The heritability ratio is used to determine the ratio between various additives variance from genetic variance, the ratio of narrow sense heritability and broad sense heritability of protein content was 81%.

Table 3. Analysis of variance combining ability character weight 10 seed, number of seeds per pod, number of pods per bunch, number of pods per plant, and yield in cowpea

SV	W100S (g)	SP	PB	PP	Y (g)
REP	1.31	1.37	0.01	58.69	46.3
Cross	5.59	4.41	0.07	72.61	88.64
GCA	28.29**	20.88**	0.16*	148.77ns	44.87ns
SCA	0.60ns	0.68ns	0.04ns	70.23ns	121.94ns
Reciprocal	1.49ns	1.55ns	0.07ns	44.53ns	72.86ns
Residuals	2.82	0.94	0.10	48.74	44.25

Note: W100S: weight of 100 seed, PB: number of pods per bunch, PP: number of pods per plant, SP: number of seeds per pod: , Y: yield,* significant at the 0.05 probability level, **significant at the 0.01 probability level, ns not significant, GCA: General combining ability, SCA: Specific combining ability

Table 4. Analysis of variance combining ability protein content in cowpea

SV	PC
REP	1.90
Cross	5.26
GCA	15.06**
SCA	1.34ns
Reciprocal	-
Residuals	1.29

Note: Protein content: PC, * significant at the 0.05 probability level, **significant at the 0.01 probability level, ns not significant, GCA: General combining ability, SCA: Specific combining ability

Table 5. Component of variance and heritability weigh of 100 seed, number of seeds per pod, number of pods per bunch, number of pods per plant, and yield per plant

Component	SP	W100S (g)	PB	PP	Y(g)
σ^2_e	0.31	0.94	0.032	16.25	14.75
σ^2_g	20.81	28.23	0.153	142.00	33.18
σ^2_s	0.22	-0.20	0.004	32.13	63.80
σ^2_r	0.62	0.27	0.017	14.14	29.05
σ^2_A	41.62	56.47	0.306	284.01	66.37
σ^2_D	0.22	-0.20	0.004	32.13	63.80
h^2_{ns}	0.99	0.99	0.894	0.85	0.45
h^2_{bs}	0.99	0.98	0.906	0.95	0.89
h^2_{ns}/h^2_{bs}	0.99	1.00	0.990	0.90	0.51

Note: W100S: weight of 100 seed, PB: number of pods per bunch, PP: number of pods per plant, SP: Number of seeds per pod, Y: yield per plant, σ^2e: environmental variance, σ^2g: GCA variance, σ^2s: SCA variance, σ^2r: reciprocal variance, σ^2A: additive variance, σ^2D: dominant variance, h^2bs: broad-sense heritability, h^2ns: narrow-sense heritability

Table 6. Component of variance and heritability protein content character

Component	PC
σ^2_e	0.43
σ^2_g	1.96
σ^2_s	0.91
σ^2_r	-
σ^2_A	3.92
σ^2_D	0.91
h^2_{ns}	0.745
h^2_{bs}	0.918
h^2_{ns}/h^2_{bs}	0.81

Note: PC: protein content, σ^2e: environmental variance, σ^2g:GCA variance, σ^2s: SCA variance, σ^2r:reciprocal variance, σ^2A: additive variance, σ^2D: dominant variance, h^2bs: broad-sense heritability, h^2ns: narrow-sense heritability

Discussion

Inheritance of trait involves additive genes effect and non-additive genes effect can be known through the variance of general combining ability (GCA) and specific combining ability (SCA) (Falconer dan Mackay 1996). The inheritance of traits in self-pollinating plants usually uses the Griffing Diallel method with a random model because it is only to see how inheritance the observed traits different from crossing pollination plants which are more specifically to forming hybrid genotypes so that the traits used have been evaluated especially for traits that support high yields, for this study the diallel Griffing method was used to evaluate parents line and the combination of best crosses (Griffing 1956).

Diallel random model, analysis showing general combining ability significantly indicates that traits are more influenced by additive gene effect, the analysis that showing significant specific combining ability indicates traits are more influenced by non-additive gene effect. Traits that have a greater general combining ability variance than specific combining ability are thought to be more influenced by additive genes (Fasahat et al. 2016), the traits with general combining ability are indicated traits more influenced by additive gene effect, which is weight of 100 seed, number of seeds per pod and number of pods per bunch are different from the results obtained on peanuts (Neya et al. 2017) the weight of 100 seeds is influenced by the effects of non-additive genes Similar results were reported by Idahosa and Alika (2013) in cowpea for 100 characters of seed weight which were influenced by additive and non additive effects. The pods reported by Neya et al. (2017) were more influenced by the effects of additive genes obtained from the results of GCA / SCA ratios greater than 1 while in the Idahosa and Alika (2013) results obtained that the number of seeds per pod was influenced by the additive gene effect and the non-additive that can be predicted from significant value of the general combining ability and specific combining ability according to Medici et al. (2004) significant general combining ability indicates that at least one of the results of the crossing affected by the effects of additive genes while significant specific combining ability indicates that at least one of the elders affected by the effect of a non-additive gene or the observed traits affected by more than one locus.

The number of pods per plant and yield per plant did not obtain significant on all components, indicating that there was no difference between the lines of the plants. Seed protein content significant in general combining ability and not significant in specific combining ability indicate that character is influenced by additive gene effect, which is different from the results obtained by Idahosa and Alika (2013) in Phaseolus vulgaris L. for protein characters affected by additive and non-additive gene effect but in peanuts (Layrisse et al. 1980) protein content was influencing by general combining ability than specific combining ability even though there are few maternal effects.

Heritability values explain the genetic proportions of the phenotype. High heritability values indicate wide diversity of the characters, making it possible to make selections (Junaid et al. 2014). The value of heritability consists of broad sense of heritability and narrow sense of heritability. Broad-sense heritability is interpreted that a character is influenced by the dominant gene's effect while narrow sense heritability indicates a character influenced by additive gene effect. A character can be used as a character selection if it has a high value of narrow heritability because the possibility of the character will be inherited from the next generation (Sujiprihati et al. 2002).

In this study the value of broad sense heritability and narrow sense heritability are high categories because more than 50% (Table 5 dan Table 6) (Syukur et al. 2010) on the character of 100 seeds weight, number of seeds per pod, number of pods per bunch, number of pods per plant and seed protein content so that the character has the potential to be used as character selection because it is controlled by additive gene effect (Sujiprihati et al. 2002) that supported by the h^2ns /h^2bs ratio (Table 5 and Table 6) which approaches 100% of these characters with the highest ratio of 100 seeds. The heritability ratio is one of the important parameters because it explains the proportion of variance additives to the total variance as stated by Saputra et al. (2014) if the value of the heritability ratio bigger or close to 100%, then a character has a higher proportion of additives.

The results obtained provide information about the relationship between general combining ability and the value of heritability. The effect of general combining power is very important to develop pure line from the crosses, while the high heritability value is an indicator that has a high diversity character, will be effective to selection in the F2 generation until F6 generation use pedigree selection method. These results support breeding in cowpea, because cowpea is selfing-pollinated plants so that they will be more effective if developed with homozygous genetic arrangements. The higher the homozygosity of a genotype, the additive genes can be fixed. It is expected that with the fixation of additive genes it will be obtained by individuals with segregation that have higher protein content and yield components than the average value of the parent value, this phenomenon is transgressive segregation namely heterosis phenomenon in segregation of self-pollinated plants.

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