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ESTIMATION OF GENETIC PARAMETERS AND SELECTION OF SOYBEAN (*Glycine max* L. Merrill) LINES DERIVED FROM SINGLE SEED DESCENT (SSD) METHODE FOR LOW LIGHT INTENSITY TOLERANCE

Wage Ratna Rohaeni¹, Trikoesoemaningtyas² and Desta Wirnas²

¹Master degree student of Plant Breeding and Biotechnology Major, Bogor Agricultural University

²Lecturer of Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University

ABSTRACT

This research was designed to estimate genetic parameters and to get inbred lines low light intensity tolerance in soybean by using single seed design (SSD) based on agronomic character and tolerance index. RILs F6 derived from hybridization between Ceneng and Godek were used in this research. The research reveal that low light intensity stress giving effect in decreasing in flowering ability, number of productive branch, number of productive node, number of productive pod, and yield. In addition, low light intensity treatment made the stem has abnormal elongation, increasing in number of empty pod, and 100 grain weight. Under low light intensity, yield productivity decrease until 32.42% than control (full light intensity). The highest yield productivity under low light intensity condition was 1.74 ton/ha. While in under control condition, yield productivity can be 2.62 ton/ha. Genetic variability was observed by using combined analysis of variance. Light intensity and genotypes treatment was significant effect in all observed characters. The highest genetic variance (σ^2_g) was in plant height character, but in number of productive pod was the highest phenotype variance (σ^2_p), environmental variance (σ^2_e), and also G x E interaction variance ($\sigma^2_{g \times e}$). Number of heritability in plant height, empty pod, and 100 grain weight were included high criteria of heritability, number of productive branch, productive node, and pod productive were included medium criteria. While in diameter stem and weight grain/plant character were included low criteria. Index toleran and relative value methode was used to select genotypes which have low light intensity tolerance trait. 20 genotypes which has low light intensity tolerance trait were selected by using both methodes. Means value of productivity by index tolerance is 1.37 ton/ha and by relative value is 1.31 ton/ha. In our research we got productivity value reach out 2.62 ton/ha from control condition.

Keywords: soybean, low light intensity, Single Seed Decent, genetics parameters, tolerance index, selection

INTRODUCTION

Soybean is a raw materials in various foods which is important source of protein in Indonesia such as fermented soybean cake (tempe), tofu, soysouce, soybean milk and others. National soybean consumption reaches about 1.803 million tons. Indonesia soybean production in 1992 achieved 1.87 million tons from a total harvest area of 1.67 millions Ha area, however the production decreased year by year. In 2007 the production only reached 0.59 millions ton from a total harvest area of 0.46 millions ha area. National program to increase soybean production has been successful in increasing soybean production in 2008 by 31.05% to 0.776 million tons from a total harvest area of 0.592 million area and in 2009 the production is expected to increase by 18.78% to 0.992 million tons from a total harvest area of 0.638 million Ha area (Departement of agriculture of Indonesia, 2009). The data shows that the reduction of soybean production is mainly due to decrease in harvest area.

The objective of the National Soybean Program is to improve soybean productivity by adoption of superior varieties and to increase soybean production area by utilization of underutilized land. One of such area is land under canopy which can be cultivated with soybean as intercrop. The total land area used for rubber plantation in 2008 was about 3.47 Ha, of which 30% is young rubber trees that can be intercropped with soybean (Departement of agriculture of Indonesia, 2008).

Cultivation of soybean as intercrop with estate crop have many constraint, namely low light intensity, drought and soil acidity. Soybean grown under shading condition become etiolated, inadequate chlorophyll content reducing photosynthetic process. Insufficient in photosyntat which were allocated into pod caused many pod was empty (Sopandie *et al.*, 2006). The percentage of empty pod character had significant negative correlation to weigth of seed/pant (Wirnas, 2006). Therefore, there is a need for a breeding program to develop a new soybean variety which is tolerant to low light intensity that can grow well under shading condition. In the past research, Ceneng and Godek was the tolerance genotype to low light intensity (Sopandie *et al.*, 2002; Sopandie *et al.*, 2006). This genotype was good as parental for create tolerance variety.

The first stage in breeding soybean is to develop population with high variability. A population of F6 inbred lines have been develop at the Department of Agornomy and Horticulture, through a single seed decent (SSD) method since 2006. Single seed decent is a method to advance segregating population after hibridization by harvesting only one seed per plant to be planted for the next generation. Selection is delayed untill the genotypes reaching homozygosity (Phoelman, 1995).

Increasing productivity is a main purpose in soybean which can be achieved through selection to improve yield potential. For intercropping with plantation crops, the soybean varietiy should also have tolerance to low light intensity. Selection for tolerance to low light intensity requires information on characters of adaptation and the inheritance. The objectives of this research were to estimate genetic parameters of tolerance to inheritance to be in seleciton of soybean lines derived from SSD.

MATERIALS AND METHODS

The study was conducted in the Cikabayan experiment station, the University Farm, Bogor Agricultural University from March to May 2009. The genetic materials used are 102 RILs F6 soybean derived from single seed descent (SSD) methode and the two parentals Ceneng (tolerant) and Godek (sensitive). The lines were grown udner low light intensity and control/open condition.

Nested design was used in this experiment with genotypes and light intensity as treatment factors. All genotypes were planted under low light intensity condition (50% light intensity) and full light intensity condition (as a control) with 3 replications. The 50% light intensity was designed by using Paraneet house. The seeds of genotype were planted one seed/hole. Each genotype consisted of 18 plants/replicate. Organic fertilizer 2 ton/ha and 1 ton/ha Ca were aplied 3 week before planting. Fertilizers consisted of 100 kg/ha, SP36 : 200 kg/ha, KCl : 150 kg/ha) were applied at first planting. Insecticides were applied when needed.

Obervatoin was made on 80 % flowering time, stem diameter, plant height, number of productive branch, productive node, productive pod, empty pod, 100 dry grain weight, grain weight/plant, and grain weight/population. Data were collected during flowering time and harvest time. Data were taken from 4 sample plants per genotype in each replication. Experimental data were analyzed using PROC ANOVA (analysis of variance) and Duncan's multiple range tests were used for mean comparison, Barlett analysis for determine homogeneity of variance was done using Minitab programme.

Table 1. Analysis of variance (Annichiarico, 2002)

Source of variance	df	MS	E(MS)
Replication	r-1	-	-
Genotype	g-1	M2	$\sigma^2_e + r(\sigma^2_g)$
Error	r(g-1)	M1	σ^2_e

Estimation of variances from analysis of variance table,

$$\sigma^2_G = (M3 - M2) / r$$

$$\sigma^2_e = M1$$

$$\sigma^2_p = \sigma^2_G + \sigma^2_e$$

Barlett's analysis was used to check homogeneity of variance. If P-Value > 0.01 then the data were not heterogenous ($\tau = 0$). According to Petersen (1994) if the data were normal and the variance was not heterogenous, data can be proceed with combined analysis.

Table 2. Combined analysis of variance (Annichiarico, 2002)

Source of variance	df	MS	E(MS)
Light intensity	(l-1)	-	-
Replication	l(r-1)	-	-
Genotype	(g-1)	M ₃	$\sigma^2_e + r\sigma^2_{gl} + rl\sigma^2_g$
G x E	(g-1)(l-1)	M ₂	$\sigma^2_e + r\sigma^2_{gl}$
Error	l(r-1)(g-1)	M ₁	σ^2_e

Estimation of variances from combined analysis of variance table,

$$\sigma^2_G = (M3 - M2) / rl$$

$$\sigma^2_{GxL} = (M2 - M1) / r$$

$$\sigma^2_e = M1$$

$$\sigma^2_p = \sigma^2_G + \sigma^2_{GxL} + \sigma^2_e$$

$$H^2_{bs} = \sigma^2_G / \sigma^2_p \times 100 \%$$

$$\text{Tolerance index} = \frac{(Y_y \times Y_x)}{\bar{Y}_x}$$

note : Y_y = yield in shading condition, Y_x = yield in control condition

\bar{Y}_x = mean of yield in control condition

RESULTS AND DISCUSSION

The genetic material evaluated in this study are recombinant inbred lines (RILs F6 of soybean (*Glycine max* L. Merrill) derived from single seed descent (SSD). Soybean grown under low light intensity condition usually cannot produce good flower. Normally soybean start flowering at 5-6 WAP (week after planting). The soybean lines evaluated under low light intensity required longer time for flowering, about 5-6 days longer than under full light condition, that were 6-7 WAP. According to Daubenmire (1974) physiology of plants live under low light incline to have longer vegetative period. The because plants have to avoid low light intensity stress. Ephratt (1993) also stated that the light intensity influenced flowering stage on soybean.

There are significant difference among soybean lines in agronomic characters grown under full light intensity. Similarly, difference among lines was also significant for agronomic characters, namely stem diameter, plant height, number of productive branch and number of productive node under low light intensity (Table 3). Low light intensity reduced stem diameters by 23.75% and increased plant height by 39.42% which caused many of the F6 lines became prone to lodging. The F6 soybean lines from SSD showed lower.

Table 3. Analysis of variance of agronomic characters and yield components of soybean grown under low light intensity and full light intensity condition (control)

Characters	Low light intensity		Full light intensity	
	MS Error	MS Genotype	MS Error	MS Genotype
Stem diameter	1.31	1.85*	0.40	0.86**
Plant height	250.57	771.39**	75.70	261.64**
Σ productive branch	0.59	1.37**	0.54	1.34**
Σ productive node	67.62	134.07**	71.36	168.22**
Σ productive pod	358.81	689.19**	306.43	694.27**
Σ empty pod	3.35	5.487**	2.38	4.24**
100 grain weight	0.05	2.17**	0.061	2.02**
Grain weight/plant	6.48	14.96**	12.13	18.12**

Note : *significant at $P = 0.05$, **significant at $P = 0.01$

Table 4. Agronomic characters of soybean lines derived from SSD under full light and low light intensity

Genotype	Stem diameter (mm)	Plant height (cm)	Σ productive branch	Σ productive node
Low light intensity condition (Shading treatment)				
RILs F6 SSD	4.2±0.8	105.2±16.2	3.9±0.7	30.1±6.5
Ceneng	4.0±0.8	111.6±18.7	3.2±0.9	24.8±8.8
Godek	4.0±0.4	110.9±12.9	3.3±0.7	24.7±2.9
Full light intensity condition (control)				
RILs F6 SSD	5.4±0.5	76.5.2±9.4	4.3±0.7	35.1±7.5
Ceneng	5.5±2.8	86.7±42.0	3.8±2.0	29.17±14.5
Godek	5.1±2.2	70.0±31.3	5.8±2.0	39.33±13.6
% change of value for agronomic characters in low light intensity than control				
Mean	-23.75	39.42	-25.18	-23.17

Table 5. Yield components of soybean lines derived from SSD under full light and low light intensity

Genotype	Σ productive pod	Σ empty pod	100 grain weight (g)	Grain weight/plant (g)
Low light intensity condition (Shading treatment)				
RILs F6 SSD	59.4 \pm 15.2	2.9 \pm 1.4	6.8 \pm 0.9	7.8 \pm 2.3
Ceneng	53.0 \pm 21.7	2.5 \pm 1.2	7.1 \pm 0.1	7.8 \pm 3.3
Godek	52.7 \pm 6.3	2.5 \pm 1.1	7.4 \pm 0.1	9.0 \pm 3.0
Full light intensity condition (control treatment)				
RILs F6 SSD	86.6 \pm 15.3	2.3 \pm 1.2	6.6 \pm 0.8	12.3 \pm 2.4
Ceneng	74.5 \pm 38.2	0.9 \pm 0.7	6.9 \pm 0.2	12.4 \pm 2.9
Godek	85.2 \pm 31.7	1.58 \pm 0.6	7.0 \pm 0.2	11.7 \pm 6.1
% change of value for agronomic characters in low light intensity than control				
Mean	-32.97	65.27	3.90	-32.42

(Table 4). Shading effect reducing value of stem diameter, number of productive branch, productive node, productive pod, and grain weight/plant. Stem diameter decrease approximately until 23.75% than normal, number of productive branch at 25.18%, number of productive node at 23.17%, number of productive pod at 32.97%, and grain weight/plant at 32.42%. Meanwhile, shading effect made the stem has abnormal in elongation, increasing in number of empty pod, and 100 grain weight. Height plant character increase approximately until 39.42% than normal, 100 grain weight at 3.90%, and number of empty pod at 65.27%. This result was supported by the research which have been done by Sopandie *et al.* (2006) that shading effect made low photosintate transfortation to the pod. Thus matter was influence increasing of empty pod, decreasing number of productive pod and grain weight.

Normality distribution of RILs F6 SSD population

There are differences of normality distribution by using Shapiro-Wilkins test between shading and control condition. Normality distribution for weight grain/plant, number of productive pod, number of productive node, number of productive branch, and plant height under shading (a) and control condition (b) can be looked in normality distribution mapping in figure 1 and 2.

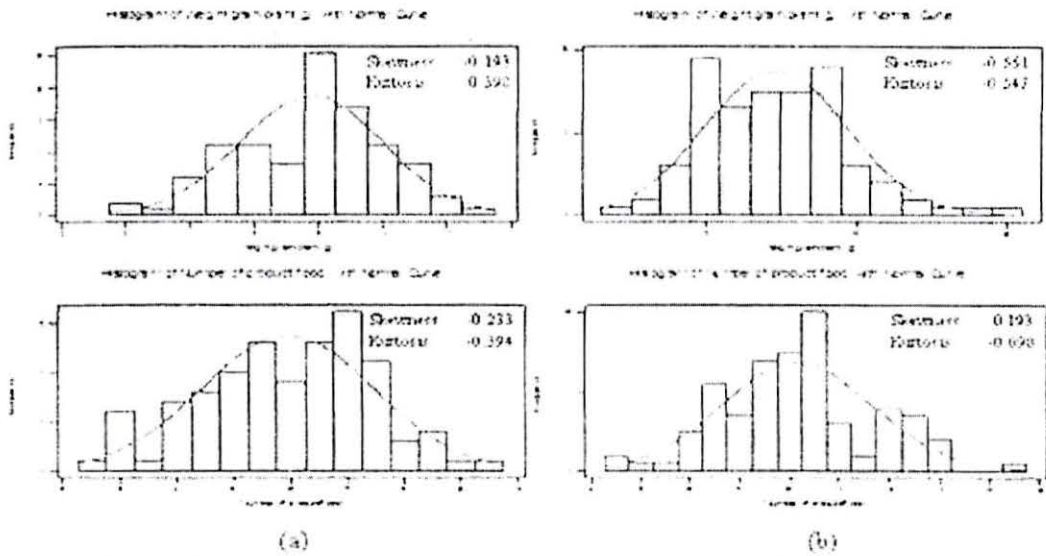


Figure 1. Normality distribution curve of RILs F6 SSD population for weight grain and number of productive character (a : shading condition, b: control)

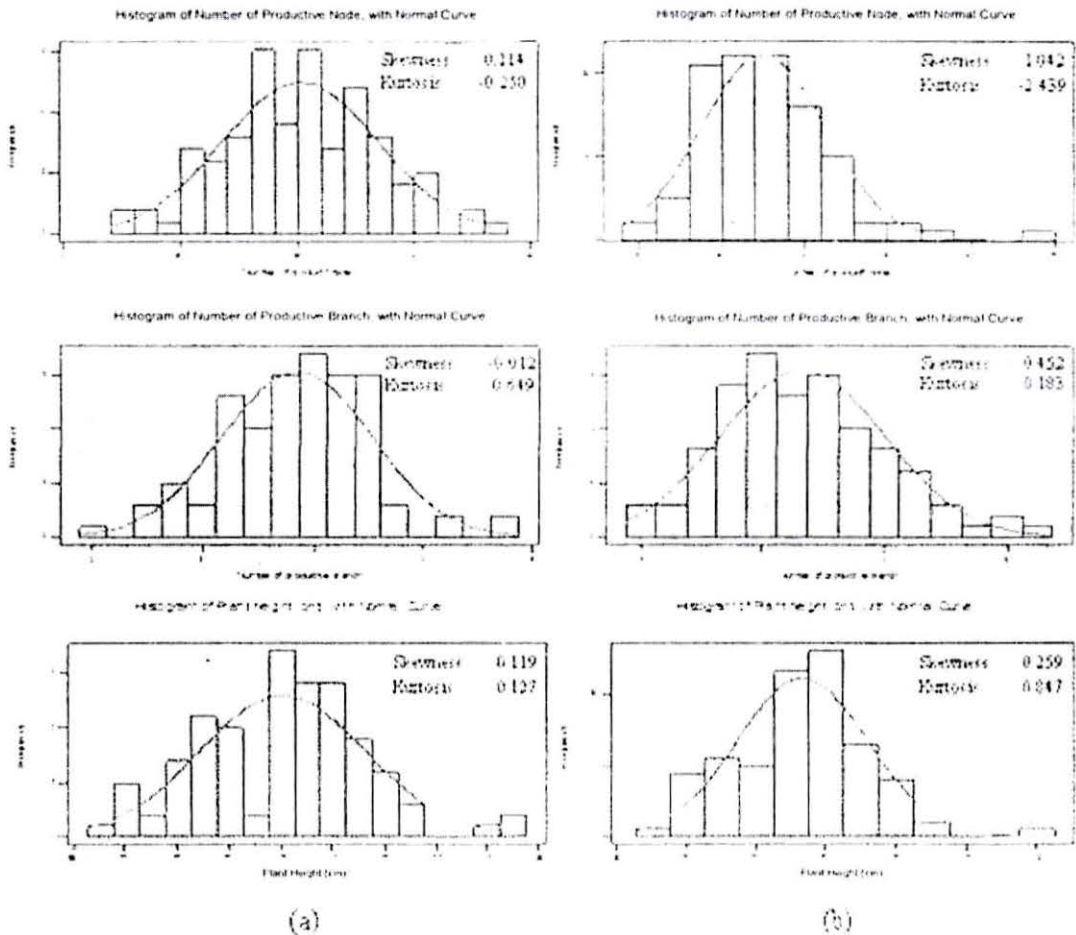


Figure 2. Normality distribution curve of RILs F6 SSD population for number of productive node, number of productive branch and plant height (a : shading condition, b: control condition)

Mostly of the characters have number of skewness < 0.5 and it's means the data was closely normal distribution, so it will support to the unrefraction result of selection proses. Bari (1998) said that abnormal data distribution is very influence to selection process for the next generation result. Abnormal data distribution can be caused by epistasis, environmental, or GxE interaction (Roy, 2000).

Genetic variance estimation and heritability value

Table 6. Genetic variance estimation in shading and control condition

Character	Shading condition				Control condition			
	σ^2_g	σ^2_e	σ^2_p	H^2_{bs}	σ^2_g	σ^2_e	σ^2_p	H^2_{bs}
Stem diameter	0.18	1.31	1.49	12.08	0.15	0.4	0.55	27.71
Plant height	173.61	250.57	424.18	40.93	61.98	75.7	137.68	45.02
Σ productive branch	0.26	0.59	0.85	30.59	0.27	0.54	0.81	33.06
Σ productive node	22.15	67.62	89.77	24.67	32.29	71.36	103.65	31.15
Σ productive pod	110.13	358.81	468.94	23.48	129.28	306.43	435.71	29.67
Σ empty pod	0.71	3.35	4.06	17.54	0.62	2.38	3.00	20.67
100 grain weight	0.71	0.05	0.75	93.93	0.65	0.061	0.71	91.46
Grain weight/plant	2.83	6.48	9.31	30.37	2.00	12.13	14.13	14.13

Table 7. Genetic variance estimation from combined analysis of variance

Character	σ^2_g	σ^2_{gxe}	σ^2_e	σ^2_p	H^2_{bs}
Plant height	71.67	43.75	174.42	122.61	58.45
Σ productive branch	0.18	0.06	1.62	0.48	36.93
Σ productive node	11.51	13.41	74.88	30.70	37.50
Σ productive pod	37.10	83.66	343.74	136.21	27.23
100 grain weight	0.50	0.18	0.05	0.60	83.81
Grain weight/plant	0.55	1.83	9.18	2.99	18.22

Notes: σ^2_g : genotype variance, σ^2_{gxe} : GxL variance, σ^2_e : environment variance, σ^2_p : phenotype variance, H^2_{bs} : heritabilitas broad sense

Genetic variance analysis result difference value of heritability between shading condition and control condition (table 6). Under control condition, several characters has H^2_{bs} value disposed higher than under shading condition. 100 grain weight was the highest heritability value, 93.93% in shading condition and 91.46% in control condition. This differences result give information that if we want to selecting lines of genotype that have some target traits that we want, the selection must be done in target condition because selection will be effective if the plants treated on target environment (Roy, 2000).

Table 7 show the result of combined analysis of variance. The data that was collected from shading treatment and control treatment were checked by Barlett's analysis to know the homogeneity of it variance before we combining analysis of variance. Unlike the other characters, stem diameter and number of empty pod couldn't proceed by combining anlysis to get GxE interaction. The test GxE interaction gives an indication of wheter or not the treatments behave the same from one location to another. All characters shown significant GxE interaction, except in number of productive branch. Value of heritability can be identified from this analysis. The highest H^2_{bs} was in 100 grain weight up to 83.81%. It is mean that 100 grain weight was insignificant influenced by environment and also GxE interaction varians

RILs F6 SSD Selection

Table 8. Selected genotypes by using Index tolerance and relative value method

No.	Genotype	Index tolerance	Productivity (kg/ha)	Genotype	Relative value	Productivity (kg/ha)
1	8	1.07	1610.56	8	0.90	1610.56
2	10	0.87	1266.67	12	0.91	1527.83
3	12	0.95	1527.83	14	0.95	1366.30
4	21	1.06	1450.33	15	1.12	1469.26
5	22	0.92	1343.78	18	1.05	1401.22
6	31	0.85	1388.33	21	1.09	1450.33
7	34	1.05	1571.78	25	0.98	1334.22
8	35	0.90	1229.17	26	1.18	1402.67
9	47	0.85	1104.56	29	1.01	1243.00
10	49	0.85	1554.00	30	0.87	1388.33
11	62	0.95	1357.00	31	1.00	1018.44
12	79	0.86	1282.17	34	1.37	1360.78
13	80	0.85	1232.11	37	1.05	1138.89
14	81	1.34	1403.56	40	0.88	1268.56
15	85	0.87	1193.78	42	1.02	1246.44
16	87	0.84	1197.78	46	0.85	1154.19
17	89	0.92	1337.33	48	0.87	1260.00
18	96	1.08	1462.33	49	0.85	1405.67
19	101	1.04	1215.85	50	0.89	1021.00
20	102	1.10	1743.11	55	1.02	1076.17
Means			1373.60			1307.19

Index tolerance and relative value method was used to select genotypes which have low light intensity tolerance trait. 20 genotypes which has low light intensity tolerance trait were selected by using both methods. Based on table 8, the genotype that has high index tolerance not always has high relative value. Means value of productivity in selected genotype from index tolerance method was higher than relative value method, but the difference looked not significant. Means value of productivity by index tolerance is 1373.60 kg/ha ~ 1.37 ton/ha and by relative value is 1307.19 kg/ha ~ 1.31 ton/ha, but we got productivity value reach out 2.62 ton/ha from control condition. In addition, the genotype that has relative value > 1 means it genotype under shading treatment has yield ability more than control. But, it not conducted in index tolerance method.

Development of good adaptation variety for abiotic stress was by looking middle harvesting age, resistance to pest and pathogen, good agronomic characters, and good quality of seed. The plant-ideotype from the all selected genotype that have good adaptation for shading stress is has harvesting age at 56-67 days, growth type semi-eterminate, plant height 84.51 - 149.11 cm, number of productive branch included much sought (3-6 branches), strong branch (branch diameter 3.5-5.0 cm), middle size seed (7.17 /100 grain), and seed color mostly dark color (black, dark brown, and green).

CONCLUSION

Low light intensity stress (shading condition) giving effect in decreasing in flowering ability, number of productive branch, number of productive node, number of productive pod, and yield. Shading treatment made the stem has abnormal elongation,

increasing in number of empty pod, and 100 grain weight. Number of heritability 100 grain weight was the highest. Means value of productivity by index tolerance is 1.37 ton/ha and by relative value is 1.31 ton/ha. Productivity value under control condition reach out 2.62 ton/ha from control condition.

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