Journal of Agriculture and Environmental Sciences
December 2016, Vol. 5, No. 2, pp. 47-55
ISSN: 2334-2404 (Print), 2334-2412 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jaes.v5n2a6
URL: https://doi.org/10.15640/jaes.v5n2a6

Performance F5 Population based on Selection Marker by Bulk Segregant Analysis on Acid Soil

Arvita Netti Sihaloho[1] & Robert Harianja[2]

Abstract

The objective of this research was to obtain information about growth and production F5 population markers tolerant Al selected in acid soil condition. For determinating markers linked to Al tolerant character and phenotyping, F2 population by crossing Argomulyo (sensitive) with Tanggamus (tolerant) and both of parent were planted in nutrient culture. Based on phenotyping root length was used for markers selection by bulk segregation analysis. F4 population was selected based on yield of plant and continued markers selection. The result shown that selection criteria in F4 population was done to seed weight seed by plant giving to repair middle value all observed characters except 100 seeds weight character was negative. Selection used molecular markers obtained genetic progress in F4 population especially to seed weight by plant character. The selection used markers produced 20 genotypes linked acid soil markers OPH-12-1200. Based on selection of yield character and markers, 30 genotypes were planted and obtained that vegetative growth and production components of genotypes linked tolerant marker better than genotypes no linked tolerant marker. There were 6 genotypes linked tolerant markers obtained, had seed weight per plant and seeds weight 100 higher than tolerant parent Tanggamus on acid soil.

Keywords: phenotyping, tolerant, direct selection, linked marker

1. Introduction

The formation of varieties is tolerant acid soil done through the plant breeding. One of the steps in process formation of tolerant acid soil varieties is selection. Selection of soybean genotypes to obtain lines can be conducted through selection directly or indirectly through secondary character or molecular markers. Activity selection to breeding conventionally can be accelerated if it can in synergy with technology molecular markers known as Marker Assisted Selection (MAS). Activity selection of becoming more effective and efficient with MAS because selection was based only on plant genetic trait, not influenced by environmental factor (Forster et al. 2000).

Efficiency and effectiveness of a method selection is measured using selection response. Selection response is determined by amount diversity within population will be selected and heritability character that become the purpose of selection (Suprapto 2007; Barman and Borah 2012). Selection response can be used as a clue in the determination of selection activity. If the response a character selection value is high that means big opportunities to undertake the improvement of the characters through selection. In contrast if selection response value is low, so selection activity on desirable characters can be conducted in once generation to form population that uniforms or activity selection can be stopped because of the improvement of characters to be achieved relatively low (Malik et al .2006).

[1] Lecturer of Faculty of Agriculture, Sisingamangaraja XII Tapanuli UniversityJl. Silangit Siborongborong North Tapanuli, Indonesia.
[2] Lecturer of Department of Biology, Faculty of Mathematics and Natural Science, Sisingamangaraja XII Tapanuli UniversityJl. Silangit Siborongborong North Tapanuli, Indonesia.

Molecular markers can be used as a tool the indirectly selection, because this molecular markers has several advantages that is to start in early generation and uninfluenced by the environment so as to have the value of thought heritability almost 100%. One of molecular markers that have been used in plant breeding plant is RAPD (Random Amplified Polimorphic DNA). Using RAPD as a tool selection have been conducted, as by Youssef et al. (2010) in rice drought tolerant and Wirnas et al. (2011) in soybean shade tolerant.

Selection method molecular markers can use a method of Bulk Segregant Analysis (BSA) that is fast can select markers linked to character of tolerance Al in acid soil. Bulk sergeant analysis based on comparisons between two DNA bulks, each consisting of DNA from individuals show extreme the phenotype, namely tolerant and sensitive of a certain properties in sergeant population (Tabor et al .2000). Using of markers RAPD which is combined with the methods Sergeant Analysis (BSA) has been used to select markers linked to a character who desirable, as *Lactic sativa* (Kesseli et al.1994), soybean shade tolerant (Trikoesoemaningtyas 2008) and tobacco (Zhang et al .2008).

Selection by using molecular markers can be used to suggest that selection progress by counting differensial selection value. Some of the results of research that have used marker assisted selection to select rice against deficiency phosfor (Ismail et al. 2007) and select yield character rice (Takai et al. 2005). This research was aimed to obtain F4 genotypes tolerant acid soil through selection yield and markers RAPD and information about growth and production of F5 population linked to tolerant Al markers in acid soil condition.

2. Material And Method

The research consists of three-stage experiment namely selection markers RAPD linked to Al tolerant, selection F4 population based on markers RAPD and seeds weight per plant character, test seeds weight per plant of preliminary lines F5 based on selection markers RAPD and yield. Experiments 1 conducted in Bimolecular Laboratory Department of Agronomy and Horticulture in April until June 2013, while experiment 2 conducted BB Biogen Research Garden, Cimanggu start March until May 2013 and experiment 3 conducted in acid soil Jasinga, Bogor, Indonesia.

Experiment 1: Analysis Marker RAPD Linked To Tolerant Al.

An analysis of markers RAPD linked to Al tolerant followed method of Bulk Segregant Analysis (BSA). Genetic materials used F2 population generated through crossing between Argomulyo (sensitive) with Tanggamus (tolerance variety). Fenotyping done by planted both of parent and F2 population in nutrient culture. The planting medium used is a solution of Ohki (1987) with a complete nutrient composition below 1.5 mM$Ca(NO_3)_2.4H_20$; 1.0 mM NH_4NO_3; 1.0 mMKCl; 0.4 mM $MgSO_4.7H_20$; 1.0 mM KH_2PO_4, 0.50 ppm $MnSO_4.H_2O$; 0.02 ppm $CUSO_4.5H_2$; 0.05 ppm $ZnSO_4.7H_20$; 0.50 ppm H_3BO_3; 0.01 ppm $NH_6Mo_7024.4H_20$; 0.068 mM $Fe(C_6H_707)$, pH 4.0 and Al 1.50 mM.

Selection tolerant and sensitive genotypes to stress Al done based on roots length characters. As many as 5 individuals F2 that represents genotype tolerant and 5 individual represents genotype sensitive to stress Al used to form DNA bulk tolerant and sensitive. Individuals tolerant and sensitive were selected based on a pattern to scatter. If the data roots length not spread abnormally and first distandarisasi with to scatter Z. The formula used is: $Z \geq \bar{x}$ + 3SDto line were tolerant and $Z \leq \bar{x}$ + 3SDto line were sensitive (Aluko & Oard 2004). Sample taken from leaves of both parent Tanggamus and Argomulyo as well as all individuals F2. The DNA genoms were isolated from both parent and five individuals F2 tolerant and five individuals F2 sensitive to stress Al. DNA isolated with using Red extract DNA Kit Sigma. Primary 60 RAPD selected to obtain primary linked to parent Tanggamus. The primary was selected used to bulk tolerant and sensitive and each individual tolerant and sensitive. Amplification use PCR ASTEC Thermal Cycler 707. Amplification is 45 cycles; consist of denaturation one minute at 94^0C. Annealing one minute at 36^0C, extention two minutes at 72^0C and stop PCR or post PCR seven minutes at 72^0C. The result of amplification will continue to electrophoresis.

Experiment 2: Selection in the Population F4 Based on Markers RAPD and Yield character

As many as 264 individuals of F4 population generated through crossing between Argomulyo (sensitive) with Tanggamus (tolerance variety) be planted by Single Seed Descent method (SSD) on without stress Al conditions. Seed planted one seed per hole with the planting of 30 cm x 20 cm follow standard cultivation for soybean in Indonesia. Observed character consist of yield and yield component character. Selection conducted is based on seed weight per plant. Selected lines were selected again with markers RAPD linked to Al tolerant. Method DNA isolation, amplification, the results of DNA visualization amplification same method in the first experiment. Best genotype selection high yield using 10% selection intensity analyzed using direct and indirectly selection. Differential selection being estimated using the formula:

$S = (\sum X_s/n_s) - (\sum X_0 / n_0)$ as; S = differential selection; $(\sum X_s/n_s)$ = mean selected population; $(\sum X_0/n_0)$ = mean before population selection.

Experiment 3: Preliminary Test of Yield SSD Lines F5 Based On Selection Yield Character and Markers RAPD

Genetic material used is 10 elected lines F5 based on seeds weight per plants and 20 elected lines F5 based on markers RAPD linked tolerant Al. Experiment was arranged in augmented design with treatment lines F5 SSD as many as 30 and 5 comparator varieties, namely varieties of Tanggamus, Anjasmoro, Argomulyo, Willis and Pangrango. SSD lines elected planted in a row without replication and comparator varieties planted as many as 4 replication. The distance used is planting of 30 cm x 20 cm. Observed character consisting of character of agronomy and yield component. The data obtained was analyzed to count middle value and analysis varians. If SSD lines influential significant to observed character so being done next test. In addition was also conducted test to know the differences between elected lines based on markers RAPD and seeds weight per plants with lines elected based on seeds weight per plant. Based on square of the middle value can be calculated the value of variance component and heritability observed character. The formula used is:

$$\sigma^2_E = KTe/r$$
$$\sigma^2_G = KTg - KTe$$
$$\sigma^2_P = \sigma^2_G + \sigma^2_E$$
$$h^2_{bs} = \sigma^2_G / \sigma^2_P$$

Note:

h^2_{bs} = broad sense heritability
σ^2_P=phenotype variance
σ^2_G =genotype variance

3. Result And Discussion

Experiment 1: Analysis Marker Rapd Linked To Tolerant Al.

Based on the results of research known that root growths at stress Al condition determine tolerance to stress Al. Plant was tolerance to stress Al having roots is longer and dried weight root was larger. The results of research shows that parent Tanggamus, Argomulyo, and F2 population having each root length is 24.1 cm, 33.2 cm and 25.6 - 40.1 cm. The results of analysis pattern to scatter roots length showed roots length in stress Al condition do not have normal to scatter so that data will be standardizated using Z value to obtain to scatter normal (Figure 1).

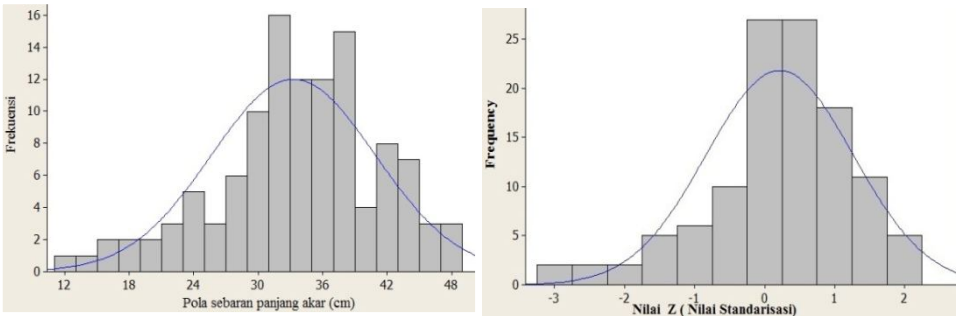

Figure 1: Distribution patterns of root length before and after standardized on population F2

Based on pattern to scatter has standardizated with to scatter Z, then elected lines was considered to be very tolerant lines stress Al, namely lines has value roots length $\geq \bar{x}$ + 3SD and lines was very sensitive namely lines has value roots length $\leq \bar{x}$ - 3SD. Individual F2 elected to form bulk tolerant and sensitive. It is present in Table 1.

Table 1.Fenotype value of root length population F_2 stress aluminum in seedling stage on nutrient culture

Number Genotype	Root length (cm)	Nilai Z	Categori
AT-SSD-91	48.5	2.07	Tolerant
AT-SSD-73	47.5	1.96	tolerant
AT-SSD-32	47	1.91	tolerant
AT-SSD-88	46	1.80	tolerant
AT-SSD-95	46	1.80	tolerant
AT-SSD-51	12	-3.16	sensitive
AT-SSD-1	12	-2.85	sensitive
AT-SSD-38	13.5	-2.55	sensitive
AT-SSD-36	15	-2.55	sensitive
AT-SSD-45	15	-2.18	sensitive

The character of tolerance to stress aluminum in the soybean plant is quantitative character; its expression is highly influenced by environmental factors. Selection to character quantitative tolerance is to be done in target to reduce influence interaction of its genotype x environment (Ceccarelli 1994). The experiment has been done in nutrient culture using characters roots length as character selection because character roots length discriminated the level of tolerance plant to stress aluminum because according to Pineros et al. (2005), Ye et al. (2011) and kochian et al. (2005) tolerant genotype have efflux mechanism Al of tip of root or having mechanism that could withstand Al in plasma membrane not to enter into the tissues and cells that can interfere with cell division.

Markers RAPD selection linked to tolerance stress Al done with methods bulk segregant analysis. The selection results of 60 RAPD for DNA tolerant parent (Tanggamus) and sensitive (Argomulyo) to stress aluminum obtained 10 primary were polymorphic. Ten primaries to amplification number of ribbon linked to tolerant parent then were selected using bulk of tolerant and sensitive genotypes of elected F2 population based on index roots length. The selection results showed one primary that consistent polymorphic on bulk tolerant and sensitive genotype (Figure 2).

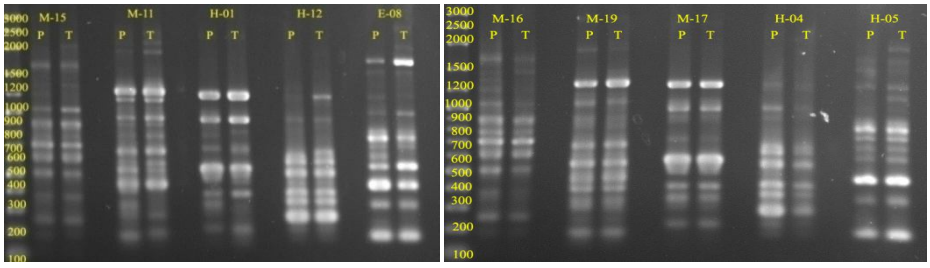

Figure 2: Selection primer RAPD polymorphic on genotype F2 bulk tolerant and sensitive

Experiment 2: Selection in the Population F4 Based On Markers RAPD and Yield Character

As many as 166 individual SSD F4 population generated through crossing between Argomulyo with Tanggamus planted on the optimum condition. The middle value of SSD F3 and F4 population and it is present in Table 2.

Table 2: Middle value performance agronomy character and yield component population SSD F_3 andF_4

Character	F3	F4
Plant height	40.0	62
Number of branch	4.1	7
Number of book	24.5	55
Total number of pods	89.3	160
Number of pithy pods	85.4	156
Number of vacuum pods	4.0	4
Seed weight per plant	14.7	28
Seed weight 100	9.1	10
Sink Size	17.1	34

The analysis showed that still there are genetic diversity in SSD F4 population to character and yield components (Table 3). Overall, genetic diversity F4 population was lower than F3population, but middle value F4 population is better than F3 population. The selection results of SSD F4 population there are in Table 4. Selection is based on seeds weight per plant. The selection results of the middle value showed improvement in all observed characters, only character seeds weight 100 of elected population smaller than early population.

Table 3: Value of genetic parameter population SSD F_3 dan F_4

Character	σ^2p		σ^2g		h^2bs	
	F3	F4	F3	F	F3	F4
Plant height	1	1			64.	69
Number of branch				4	75.	62
Number of book	1	3			55.	71
Total number of pods	23	49	18	3	81.	72
Number of pithy pods	21	46	17	3	81.	73
Number of empty pods				7	69.	48
Seed weight per plant		1			77.	34
Seed weight 100					23.	34
Sink Size		2			82.	43

The utilization of molecular markers in selection of character tolerance to stress aluminum considered can improve efficiency and progress of selection, because selection conducted with genotype so as not influenced by the environment. The selection of markers with method of bulk sergeant analysis using DNA F2 population generated through crossing between Argomulyo with Tanggamus because growth is still diverse and segregant still very high. The results of primary selection RAPD with method BSA shows that primary consistent amplificated OPH-12-1200 in individual's tolerant plants in F2 population and not on individual's sensitive. The markers OPH-12-1200 be used in as marker assisted selection in F4 generation.

Table 4: Differencial selection value based on seed weight plant^{-1} of F_4population (Argomulyo x Tanggamus) on not stress

Character	Mean begin Population	Mean selected Population	Differencial Selection (%)
Plant height	62,0 ± 12,0	63,8 ± 1,6	3,4
Number of branch	7,0 ± 2,0	9,1 ± 0,5	22,8
Number of book	55,0 ± 20,0	77,4 ± 3,0	28,9
Total number of pods	160,0 ± 70,0	49,3 ± 0,3	35,8
Number of pithy pods	156,0 ± 68,0	43,5 ± 9,9	35,9
Number of vacuum pods	4,0 ± 4,0	5,8 ± 0,9	31,0
Seed weight per plant	28,0 ± 13,0	46,9 ± 1,9	40,3
Seed weight 100	10,0 ± 2,0	9,9 ± 0,2	-0,5
Sink Size	34,0 ± 15,0	54,1 ± 2,4	37,1

The purpose of selection is to obtain an increase in frequency of genes that are desirable in next generation. The results of research showed performance of genotype F4 population observed very diverse in optimum conditions. Based on value of genetic diversity of character and heritability observed, selection criteria in F4 population are done on weight per plant seeds, because it has value of genetic diversity and heritability high. Pandini et al. (2002) and Iqbal et al. (2010) reported that number of pods per plant, number of branch per plant and weights 100 seeds can be used in plant breeding program to select yield higher of genotypes. As many as 20 lines had high yield and linked to markers RAPD OPH-12-1200 and and 10 elected lines base on seeds weight per plant tested preliminary of yield per plant in acid soil, Jasinga, Bogor, Indonesia (Table 5).

Table 5: Performance seed weight plant-1 of 30 marker selected genotypes based on Seed weight plant-1 character and marker

No	Genotype	Seed weight plant-1	Marker
1.	AT –SSD – 437	78.63	-
2.	AT –SSD – 423	67.50	+
3.	AT –SSD – 529	67.40	+
4.	AT –SSD – 503	63.91	+
5.	AT –SSD – 205	62.91	+
6.	AT –SSD – 299	51.90	+
7.	AT –SSD – 231	50.20	+
8.	AT –SSD – 552	47.55	+
9.	AT –SSD – 461	46.73	+
10.	AT –SSD – 491	46.29	+
11.	AT –SSD – 429	45.43	+
12.	AT –SSD – 541	43.79	-
13.	AT –SSD – 463	43.77	-
14.	AT –SSD – 272	43.68	-
15.	AT –SSD – 222	39.25	-
16.	AT –SSD – 263	38.08	-
17.	AT –SSD – 306	37.48	+
18.	AT –SSD – 198	37.45	-
19.	AT –SSD – 12	35.40	+
20.	AT –SSD – 256	34.56	+
21.	AT –SSD – 517	32.78	+
22.	AT –SSD – 57	31.48	-
23.	AT –SSD – 506	30.08	+
24.	AT –SSD – 33	28.95	-
25.	AT –SSD – 212	28.70	+
26.	AT –SSD – 522	27.63	-
27.	AT –SSD – 1	25.60	+
28.	AT –SSD – 281	24.50	+
29.	AT –SSD – 476	24.32	+
30.	AT –SSD – 8	22.99	+

The direct selection criteria in the population F4 was conducted on the weighting of seeds plants^{-1}, because it has value diversity of genetic and heritability is high When selection be done on best genotype obtained to improvement of mean value to all observed characters except character of weight 100 seeds was negative. It indicated that if selection done in weighting of seed plant^{-1} character so will cause disadvantage genetic of -8.3 % for weight 100 seeds character.

Experiment 3: Preliminary Test Of Yield SSD Lines F5 Based On Selection Yield Character And Markers RAPD

Based on analysis middle value of elected genotype F5 markers tolerant acid soil for character of plant height, number of productive branch per plant, number of pods pithy per plant, number of empty pods per plant, number of books productive per plant, seeds weight per plant, total amount pods, seeds weight 100, sink size and flowering age observed are between both of Tanggamus with Argomulyo (Table 6).

Table 6: Mean value of agronomy character of 30 marker selected genotypes, Argomulyo, Anjasmoro, Tanggamus, Willis and Pangrango in acid soil

Character	Genotypes selected	Variety				
		Argomulyo	Anjasmoro	Tanggamus	Willis	Pangrango
Plant height (cm)	45.04±0.9	32.08±1.0	42.15±2.9	46.35±0.8	33.77±4.6	53.05±3.2
Number of branch	3.22±0.1	.10±0.3	4.35±0.4	3.90±0.3	2.88±0.1	2.73±0.3
Number of book	27.83±0.9	17.55±0.7	24.43±3.4	33.40±3.1	22.97±1.6	24.38±4.3
Total number of pods	62.21±3.6	35.80±2.3	42.45±4.8	63.85±1.9	37.83±2.9	40.75±3.3
Number of pithy pods	58.07±3.4	31.30±2.2	38.45±5.7	59.38±2.1	38.47±4.3	34.40±4.4
Number of empty pods	3.94±0.4	4.50±2.2	2.00±1.1	3.50±0.5	1.88±0.4	4.35±1.3
Seed weight per plant (g)	10.39±0.7	7.47±0.5	8.40±0.9	12.48±0.9	7.47±0.7	7.02±0.5
Seed weight 100 (g)	11.48±0.2	12.86±0.5	11.13±0.6	10.37±0.5	10.48±0.5	11.66±0.9
Sink Size	12.88±0.9	8.57±0.4	11.38±3.5	14.74±2.0	8.83±1.0	10.49±1.4
Flowering age (day)	37.00±0.2	35.75±0.3	39.50±0.3	38.05±0.3	39.00±0.0	40.50±0.3

The analysis variance (Table 7) showed there were diversity among genotypes tested, caused by the influence of genotype, environment and interaction between genotype with environment.

Table 7: Mean square agronomy character F_5 genotype and control on acid soil

Character	Mean Square			
	Family	Genotype (G)	Control (K)	G x K
Plant height	57.14**	17.51**	305.97**	210.93**
Number of branch	0.72**	0.42**	3.05**	0.09**
Number of book	40.14**	24.63**	129.87**	130.94**
Total number of pods	469.43**	344.62**	473.55**	4072.42**
Number of pithy pods	421.26**	294.64**	484.45**	3836.34**
Number of vacuum pods	4.17tn	3.97tn	4.42tn	97.33tn
Seed weight per plant	14.69**	12.99**	20.13**	42.39**
Seed weight 100	1.81*	1.15tn	4.00**	12.47tn
Sink Size	24.12tn	23.10tn	24.82tn	51.00tn
Flowering age	3.91**	1.73**	12.83**	31.51**

The variance of genetic, environment, phenotype and heritability of each character presented in Table 8. The expected value of genetic variance was highest in character of total number pods and number of pithy pods. The value of character heritability to know to expect progress from a selection, the character is heavily influenced by genetic factors or environmental. The results of research shows that the character of higher plants, the number of the productive branches plants^{-1}, the number of pods pithy plants^{-1}, number of books productive plants^{-1}, weight seeds plants^{-1}, the total amount pods, weight of 100 seeds, sink size and age flowering observed had heritability broad sense value were high except for number of empty pods character had heritability broad sense value was low. The character had heritability broad sense value was high shows that the character more controlled by genetic factors than by environmental factors as well as genes that the character is controlled by additive gene action. According to Roy (2000) the success of the selection of very much determined by the diversity of genetic factors that is controlled.

Table 8: Thought value genetic variance and broad sense heritability F_5 selected population and Control in stress Al condition

Character	σ2p	σ2e	σ2g	h2bs	Criteria
Plant height	4.78	0.78	3.60	82.30	high
Number of branch	0.11	0.02	0.09	81.82	high
Number of book	6.16	0.83	5.33	86.56	high
Total number of pods	86.16	8.68	77.47	89.92	high
Number of pithy pods	73.66	14.68	58.98	80.08	high
Number of vacuum pods	1.11	0.83	0.23	24.89	low
Seed weight per plant	3.25	0.40	2.84	87.55	high
Weight of 100 seeds	0.29	0.14	0.15	51.72	high
Sink size	5.77	4.20	1.57	27.21	low
Flowering age	0.43	0.03	0.40	92.78	high

The results of research (Table 9) showed that vegetative growth and production components of genotype F5selected markers in acid soil have all growth observed better than genotype having no markers tolerant acid soil. Research in generation F5 indicated that there are difference in the character of agronomy genotypes linked to marker OPH-12-1100 in acid soil conditions than both parent varieties. Difference of agronomy character was on character height plants, number of productive branches per plants, total number of pods, number of pithy pods per plant, weight of 100 seeds, sinks size and aged flowering observed in genotypes in acid soil conditions. Genotypes linked to marker OPH-12-1100 having mean value are among both parent for character of height plant, number of productive branches plants^{-1}, total number of pods, number of pithy pods plants^{-1}, number empty of pods plants^{-1}, number of productive books, weight of seed plants^{-1}, weight of 100 seeds, sink size and aged flowering observed.

Table 9: Performance agronomy character genotype F_5based on selection marker in acid soil

No	Line	Agronomy Character										Marker
		1	2	3	4	5	6	7	8	9	10	
1.	AT –SSD – 429	52.4	3.6	31.0	92.6	4.6	20.8	11.5	23.5	107.2	38.0	+
2.	AT –SSD - 1	51.6	3.2	33.6	87.0	3.8	17.9	11.7	22.5	90.8	38.0	+
3.	AT –SSD – 476	57.4	3.8	36.4	98.4	3.4	16.4	10.7	22.9	101.8	39.0	+
4.	AT –SSD – 503	46.4	3.6	34.4	79.6	2.4	15.3	11.0	18.5	82.0	36.0	+
5.	AT –SSD - 8	46.0	2.8	27.2	75.8	4.6	14.0	12.5	19.1	80.4	35.0	+
6.	AT –SSD – 423	46.2	3.4	34.8	56.0	2.8	13.1	10.9	13.4	58.8	37.0	+
7.	AT –SSD – 517	41.6	3.2	22.6	59.8	0.8	12.0	10.8	13.6	60.6	38.0	+
8.	AT –SSD – 212	48.4	3.4	26.8	77.8	9.0	11.7	11.9	19.1	86.8	37.0	+
9.	AT –SSD – 491	42.6	3.4	27.0	66.8	1.6	11.6	9.4	12.4	68.4	35.0	+
10.	AT –SSD – 529	54.8	4.2	33.4	70.8	5.0	10.9	9.9	14.7	75.8	38.0	+
11.	AT –SSD – 205	48.4	2.6	34.4	63.4	2.6	10.2	10.0	13.2	66.0	38.0	+
12.	AT –SSD – 281	48.8	2.8	25.4	58.2	2.2	10.0	9.9	12.1	60.4	38.0	+
13.	AT –SSD – 461	41.6	4.6	36.2	64.0	7.0	9.9	11.6	15.8	71.0	39.0	+
14.	AT –SSD – 306	47.6	3.1	30.2	35.2	3.8	9.6	10.8	8.4	39.0	38.0	+
15.	AT –SSD – 299	42.2	2.0	29.2	35.2	4.4	9.0	11.2	8.0	39.6	33.0	+
16.	AT –SSD – 231	43.4	3.4	26.6	55.8	5.8	9.0	10.9	12.9	61.6	37.0	+
17.	AT –SSD – 506	43.2	4.4	31.4	63.6	3.8	8.7	9.1	11.8	67.4	38.0	+
18.	AT –SSD – 552	46.0	3.2	27.0	54.8	3.6	8.2	9.1	10.2	58.4	37.0	+
19.	AT –SSD - 12	47.0	3.0	25.0	51.2	2.2	8.1	9.4	9.5	53.4	38.0	+
20.	AT –SSD – 256	44.0	2.2	18.4	39.4	4.4	7.3	11.5	8.9	43.8	36.0	+
21.	AT –SSD – 522	47.2	3.0	25.4	59.6	5.8	11.0	11.3	4.0	65.4	38.0	-
22.	AT –SSD – 272	50.2	2.6	23.0	58.4	2.4	10.4	11.5	13.6	60.8	37.0	-
23.	AT –SSD - 57	47.6	3.8	24.0	49.6	2.8	10.2	10.3	10.7	52.4	37.0	-
24.	AT –SSD – 437	39.4	3.6	25.4	65.0	2.8	10.1	8.6	12.2	67.8	35.0	-
25.	AT –SSD – 541	42.8	2.8	18.0	27.4	4.2	7.3	9.9	5.7	31.6	36.0	-
26.	AT –SSD – 222	43.8	2.4	24.8	22.6	4.0	7.1	8.4	3.8	36.6	38.0	-
27.	AT –SSD – 463	49.4	4.2	27.6	47.8	3.0	6.2	11.5	12.8	50.8	36.0	-
28.	AT –SSD – 263	40.8	3.2	28.6	52.4	10.2	5.7	9.9	10.8	62.6	38.0	-
29.	AT –SSD – 198	38.6	3.2	20.6	36.2	2.8	5.3	10.2	6.6	34.8	36.0	-
30.	AT –SSD - 33	40.8	2.0	26.4	37.8	2.4	5.1	10.0	5.7	30.2	36.0	-
31.	Argomulyo	32.1	2.1	17.6	31.3	3.3	7.5	12.9	8.6	35.8	35.8	
32.	Tanggamus	46.4	3.9	33.4	59.4	3.5	12.5	10.8	14.7	62.9	38.3	

Keterangan: (1) Plant height; (2) Number of branch; (3) Number of book; (4) Number of pithy pods; (5) Number of vacuum pods; (6) Weight of seed by plant; (7) Weight of 100 seeds; (8) Sink size; (9) Total number of pods; (10) Flowering age

The middle value of agronomy character and yield component elected genotypes F5 based on markers tolerant planted in acid soil presented in Table 10. The results of research showed that the middle value of genotype linked to markers better compared with the genotype no linked to markers. It showed that a cross has been success successfully improve or change the quantitative desired associated with an increase in the weight 100 seeds of the parent tolerant (Tanggamus) that the weight of relatively small which is 8-10 g/100 seeds so genotypes selected produced in increasingly 11.48 g/100 seeds. Based on the results genotypes AT-SSD–429, AT–SSD–1, AT–SSD–476, AT–SSD–503, AT–SSD–8 and AT–SSD–423 was higher genotypes had weigh 100 seeds 11.48g/100 than parent toleranTanggamus planted in acid soil.

Table 10: Middle value of agronomy and yield character genotype F_5 linked to marker and genotype no linked to marker in acid soil

Character	Genotype F5 linked to m	Genotype F5nolinked to marke	P-Value
Plant height	46.98	44.06	0.09tn
Number of branch	3.30	3.08	0.43tn
Number of book	29.55	24.38	0.00 **
Number of pithy pods	64.27	45.68	0.00 **
Number of vacuum pods	3.89	4.04	0.86tn
Weight of seed by plant	11.69	7.84	0.00 **
Weight of 100 seeds	10.69	10.16	0.21tn
Sink size	14.53	8.59	0.01 **
Total number of pods	68.66	49.30	0.01 **
Flowering age	37.15	36.70	0.35tn

4. Conclusion

The directly selection criteria was conducted on weight seed plant^{-1} character of the population F4, Directly selection conducted on weight seed plant^{-1} character had differential selection value higher than indirectly selection. Using molecular markers selection gave genetic progress in the population especially to weight seed plant^{-1} character. The using selection of markers produced 20 genotypes linked to markers OPH-12-1100 tolerant acid soil. The planting of 30 genotype selection based on weight seed plant^{-1} character and markers obtained that the growth of vegetative and components production of genotypes linked to markers OPH-12-1100 better than genotypes no linked to markers. Based on weight 100 seeds and weight seed plant^{-1} character were higher than parent tolerant Tanggamus, obtained 6 lines linked to marker OPH-12-11

5. References

Aluko G.K., Oard J.H.2000. Evaluation of discriminant analysis as a tool for rapid identification of marker associated with drought resistance in rice. Rockefeller foundation workshop. CIMMYT.

Arsyad D.M.,AdieM.M.,KuswantoroH.2007. Perakitan Varietas Unggul Kedelai Spesifik Agroekologi. *Dalam* Sumarno, Suyamto, Widjono A, Hermanto, Kasim

Azrai M. 2006. Sinergy of molecular marker technology in corn breeding in Indonesia*J. LitbangPertanian*.25(3):81-89.

Babu R.S.K., Nar., PrasannaB.M.,H.S. Gupta. 2004. Marker Assisted Selection and Crop Improvement. Current Science Special Review. 81:607-619.

Iqbal Z., M. Arshad,.Ashraf R.,Naeem R.,MaliK M.F.,Waheed A. 2010. Genetic divergence and correlation studies of soybean [*Glycine max* (L.) Merrill.] genotypes. *Pak. J. Bot*. 42(2): 971-976.

Khlestkina E.K.,Than M.H.M.,Pestsova E.G,RoderM.S., Malyshev S.V.,

Korzun V., Borner A. 2004. Mapping of 99 new microsatellite-derived loci in rye (Secalecereale L.) including 39 expressed sequence tags. *TheorAppl Genet*. 110: 990–991

Martono B. 2009. Genetic variability, heritability, and correlation among quantitative characters of patchouli (Pogostemon sp.) derived from protoplast fussion. *J. Litri*.15(1): 9-15.

Sa'diyah N., Basoeki T.R., Saputra A., Firmansyah, UtomoS.D.2010. Genetic parameters and correlation among agronomic characters of yard-long been in F4 population derived from crosses between the lines of testacoklat x coklat putih.*J. Agrotropika*. 15(2): 73-77.

Sleeper D.A.,PoehlmanJ.M. 2006. Breeding field crops. 5th edition. Ames: Blackwell Pub Iowa.424 pp.

Semagn K., A. Bjornstad, Y. Xu 2010. The genetic dissection of quantitative traits in crops. *Elect.J. of Biotech*. 13(5): 1-45.

Sanjaya L. 2003. Amplified fragment length polymorphism marker linked to antracnoseresistenton bulk segregant analysis. *J. Hort*. 13(3): 169-179.

Terry L.I., ChaseK., JarvicT., OrfJ., MansurL.,LarkK.G.2000. Soybean quantitative trait loci for resistance to insect. *Crop Sci*. 40:375-382.

Wirnas D., SopandieD., Trikoesoemaningtyas, Sobir. 2011. Analysis of RAPD marker linked to shading stress tolerance of soybean.*J. Agro. Indo* 39(2):73-78.

Zhang H.Y., YangY.M., LiF.S., HeC.S., LiuX.Z. 2008. Screening and characterization a RAPD marker of tobacco brown-spot resistant gene. *Afric. J. of Biotech*. 7(15): 2559-2561.