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SEGREGATION PATTERN OF RESISTANCE TO SOYBEAN MOSAIC VIRUS ON TANGGAMUS X TAICHUNG CROSSED POPULATIONAT $F_{2:3}$ GENERATION

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ABSTRACT

Segregation is one of the genetic parameters used to determine the proportion of progenies of a particular phenotype The research aimed to determine the distribution pattern of gene frequency of resistance to a disease caused by *Soybean Mosaic Virus* on the $F_{2\cdot3}$generation of Tanggamus X Taichung crossed progenies. In addition to the segregation of characters resistant to the disease, the research also generated patterns of segregation for other characters namely, plant height, pod number per plant , 100 seed weight per plant and total seed weight per plant. The research was conducted at the Integrated Field Laboratory and the Laboratory of Seeds and Plant Breeding, University of Lampung, Bandar Lampung, Indonesia from June to September 2014. Conformity to a normal distribution and analysis of segregation pattern were tested using a Chi-squared test at $\alpha_{0.01}$. The results showed that resistance to Soybean mosaic virus did not spread following a normal distribution, indicating that the characters were semi-dominant.

Key words: soybean breeding, soybean mosaic virus, segregation patterns

INTRODUCTION

Low productivity in soybean (*Glycine max* [L.] Merrill) is caused by several factors including disease, such as mosaic due to *Soybean Mosaic Virus* (SMV). The disease was considered extremely important in many countries (Hill 1999), with SMV infection during vegetative phase resulting in yield loss of up to 25 %, whereas infection during the beginning of growth resulting in a yield loss reaching 90 % (Prayogo 2012). An effective way of preventing yield loss due to oybean mosaic disease is by using resistant varieties. To develop soybean varieties resistant to SMV, while maintaining high yield is by crossing two varieties having those complementary desirable characters. According Sa'diyah et al. (2016), the F_1 generation of a cross between Yellow Bean X Tanggamus, Tanggamus X Orba, and Tanggamus X Taichung were resistant to SMV. When the F_2 generation of the Tanggamus X Taichung cross was retested for SMV resistance, the progenies continued to show resistance to SMV, and the weight of seeds per plant exceeded that of the two parents (Aslichah et al. 2014, Wanda et al. 2015).

SMV resistance was controlled by a single dominant gene (Buss et al. 1985, Shi et al. 2008), or a single intermediate dominant gene (Shi et al. 2008). According to Shi et al. (2008) resistance properties were controlled by a single dominant gene for the G_1SMV, while a single intermediate dominant gene controlled for G_7 SMV. Therefore, it was necessary to study the segregation pattern of resistance to soybean mosaic disease in the $F_{2}:_{3}$ generation of Tanggamus X Taichung.

The research sought to determine the distribution pattern of gene frequency of SMV resistance at the $F_{2:3}$ generation of Tanggamus X Taichung and, evaluate the segregation on plant height, pod number per plant, 100 seed weight per plant and seed weight per plant.

MATERIALS AND METHODS

The research was conducted at the Integrated Field Laboratory and the Laboratory of Seeds and Plant Breeding at the University of Lampung, Bandar Lampung, Indonesia from June to September 2014 using a non-repetitive experimental design. Soybean $F_{2.3}$ seeds were developed through a non-reciprocal diallele mating, which crossed 10 parental lines (Barmawi et al. 2012). The F_1 generation was tested against SMV in 2013 (Sa'diyah et al. 2016) and the F_2 generation in 2015 (Wanda et al. 2014). The inoculum of SMV was isolated frm a natural source. The inoculum was augmented by infecting it on Tanggamus susceptible to SMV. The inoculum was prepared by crushing 5 mg of naturally infected leaves in 50 ml phosphate buffer solution at pH 7. Phosphate buffer was made of two solutions, Solutions A and B. Solution A consisted of 1.36 g KH_2PO_4 and solution B was 1.78 g Na_2HPO_4. $2H_2O$ dissolved in 1 L distilled water. Phosphate buffer was made by mixing 510 ml of solution A with 490 ml solution B.

The inoculum (sap extracted from the infected leaves) was used to infect sample plants. Virus inoculation was performed on soybean plants that already had fully open leaves at 7 – 10 days after planting (DAP). The leaves were sprinkled with zeolite to inflict abrasion and were sprayed with SMV suspension. After inoculation, the leaves were washed with distilled water.

Data was analysed through chi-square test for their normal distribution and segregation to test for conformation between observed and expected values (Gomez and Gomez 1995). Observations were made on each soybean plant involving variables: (1) disease severity was calculated at 42 DAP on 10 leaves of the test plants following the protocol of Campbell and Madden (Mulia 2008); (2) plant height; (3) pod number per plant ; (4) 100 seed weight per plant ; and (5) total seed weight plant.

The severity of the disease (%) was evaluated as

$$DS = \frac{\Sigma(n \times V)}{N x Z} \text{ x } 100\%$$

where DS = Disease severity

N = Number of sample plants

Z = Highest score

n = Number of sample plants per infection category

V = Score for infection category

Figure 1 presented pictures of infected leaf and scoring method in categorising the severity of the disease (Akin and Barmawi 2005).

Gene inheritance, which controlled the characters having a fitted ratio between the observed and the expected values, was considered as the number of genes which controlled the characters. When the controlling genes were simple, the $F_{2:3}$ population would match some ratios in the form of graphs as follows: (1) if with two peaks, the likelihood ratios of phenotypic segregation would be 3: 1, 9: 7, 13: 3, and 15: 1; (2) if with three peaks, the likelihood ratios would be 1: 2: 1, 9: 3: 4, 9: 6: 1, and 12: 3: 1; (3) if with more than three peaks, the likelihood ratios would be either 9: 3: 3: 1 or 6: 3: 3: 4; and (4) if with a unimodal shape, segregation would be polygenic (Snyder and David 1957).

Segregation ratios evaluated in the research was the ratio of the F_2 population since the plants came from a number of genotypes of the F_2 population. It was assumed that the selected genotypes were segregating heterozygous. Individuals which heterozygous would segregate following the pattern of F_2 segregation

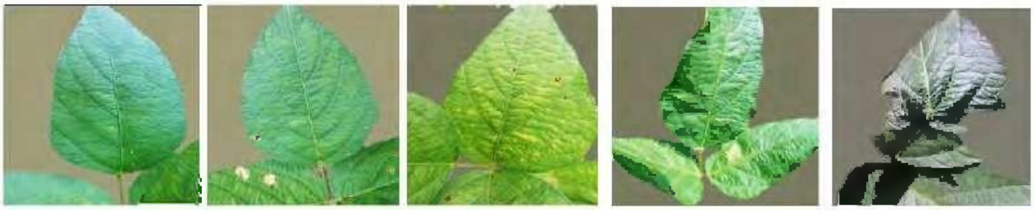

Fig. 1. Symptoms on infected leaves and their disease scoring
(A) No symptom= 0; (B) Chlorosis and decoloration on midrib= 1; (C) Mosaic with chlorosis on midrib and leaf surface= 2; (D) Heavy mosaic and chlorosis, leaf bended upward or downward= 3; (E) Leaf malformation= 4.

Resistance category based on Disease Severity (%):(Akin and Barmawi, 2005).
0 – 15 = Highly Tolerant
16 – 25 = Partially Tolerant
26 – 35 = Partially Susceptible
36 – 55 = Susceptible
56 – 100 = Highly Susceptible

RESULTS AND DISCUSSION

The severity of disease caused by SMV as measured on plant height, 100 seed weight per plant characters did not follow the normal distribution (Table 1; Figure 2-6) indicating that these characters were controlled by one or two major genes. The pattern of segregation followed the Mendelian ratio or its modification (Fehr 1987). The result corroborated with that of Wanda et al. (2014) who indicated that severity of SMV infection was in accordance to the Mendelian ratio or its modification.

Table 1. Chi-square test for the fit to the normal distribution.

No.	Character	χ^2count	χ^2table	The Frequency Distribution
1.	Severity of disease	54.15	12.59	Abnormal
2.	Plant height	116.99	18.47	Abnormal
3.	Pod number per plant	5.11**	18.47	Normal
4.	100 seed weight per plant	22.22	13.28	Abnormal
5.	Seed weight per plant	8.31**	18.47	Normal

Note: * = different at $\alpha_{0.01}$

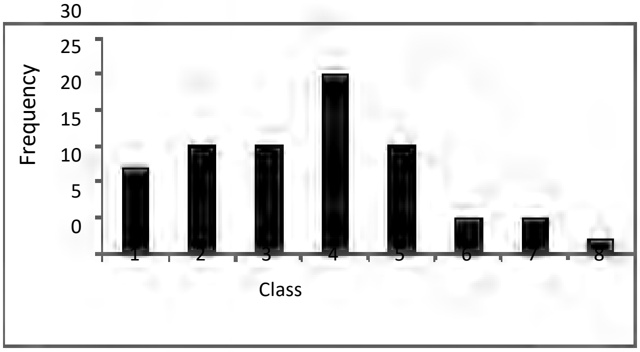

Fig. 2. The normal distribution for pod number per plant on Tanggamus X Taichungcrossed population at $F_{2:3}$ generation.

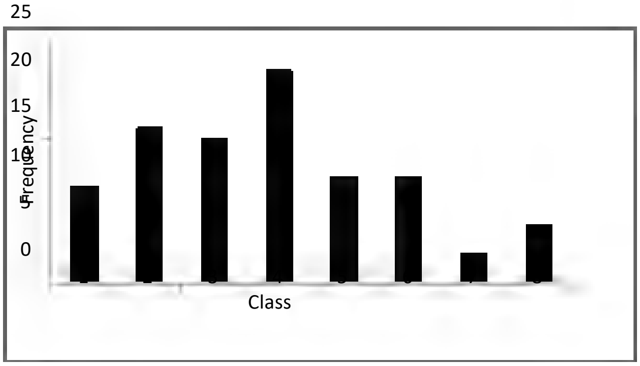

Fig. 3. The normal distribution for grain weight per plant on Tanggamus X Taichung crossed population at $F_{2:3}$ generation.

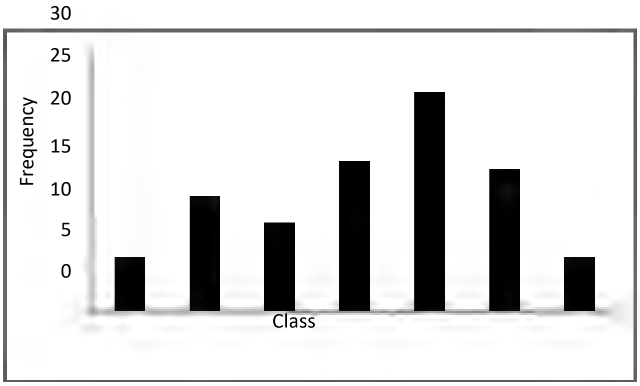

Fig. 4. The abnormal distribution with three peaks for severity of disease on Tanggamus X Taichung crossed population at $F_{2:3}$ generation.

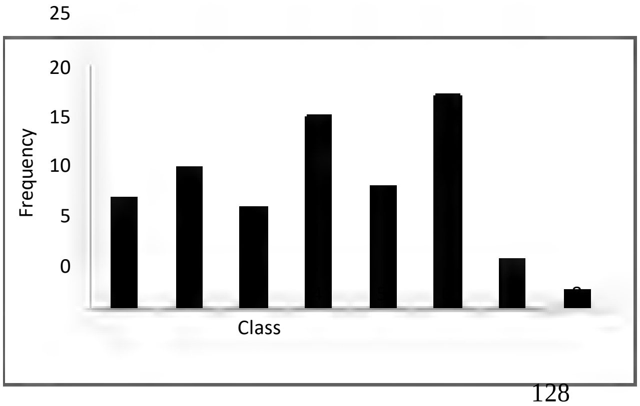

Fig. 5. The abnormal distribution with two peaks for plant height on Tanggamus X Taichung crossed population at $F_{2:3}$ generation.

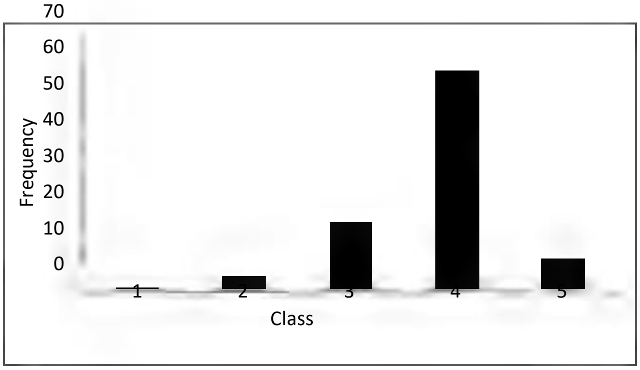

Fig. 6. The abnormal distribution with two peaks for 100 seed weight per plant on Tanggamus X Taichung crossed population at $F_{2:3}$ generation.

The segregation ratio of the severity of the SMV infection on the $F_{2:3}$ generation of Tanggamus X Taichung cross was 1: 2: 1 with of 50-75 % of augmentation suggested that the character was controlled by one dominant gene (Synder and David 1957; Table 2). The differences in the disease severity on each plant were observed in the field. Low infection rate observed on the resistant plants might be due to their ability to inhibit viral replication, contain the virus in the infected cells and prohibit the virus to infect other cells or plant tissues (Akin 2006). Agrios (1996), indicated that the infection rate showed by a high severity of the disease might be caused by substances in the cell fluid suitable for the growth and development of the SMV. The infection worsened when the virus replication was supported by environmental conditions which reduced the host capability to impede the growth of the virus.

Table 2. Chi-squared segregation ratios for the severity of disease caused by SMV infection on the Tanggamus X Taichung crossed population at $F_{2:3}$ generation.

Ratio	Observation	Expectation	χ^2count	$\chi^2\alpha_{0.01}$	P $\chi^2>\alpha_{0.01}$
Two Classes					
3:1	33 : 61	70.5 : 23.5	78.74**		<0.005
9:7	33 : 61	52.88 : 41.13	16.98**	6.64	<0.005
13:3	33 : 61	76.38 : 17.63	129.50**		<0.005
15:1	33 : 61	88.13 : 5.88	543.01**		<0.005
Three Classes					
1:2:1	20:48:26	23.5 : 47 : 23.5	0.81		0.50-0.75
9:3:4	20:48:26	52.88 : 17.63 : 23.5	73.05**	9.21	<0.005
9:6:1	20:48:26	52.88 : 35.25 : 5.88	93.99**		<0.005
12:3:1	20:48:26	70.5 : 17.63 : 5.88	68.94**		<0.005
Four Classes					
9:3:3:1	16 : 17 : 42 : 19	52.88 : 17.63 : 17.63 : 5.88	88.77**	11.35	<0.005
6:3:3:4	16 : 17 : 42 : 19	35.25 : 17.63 : 17.63 : 23.5	45.11**		<0.005

Note: **= different at $\alpha_{0.01}$

There were three selected genotypes that expressed 25% of disease severity and therefore considered as resistant. The third genotype was also selected due to high pod number and seed weight per plant and resistance to SMV infection. The severity of infection was measured from the leaf damage because the infection decreased the biochemical processes of damaged chloroplasts as well as decreased other photosynthetic pigments such as carotene and xanthophyll (Akin 2006).

In a segregating generation, if the frequency of gene which controlled a particular phenotype segregated to a normal distribution, the phenotype would be a quantitative character. On the other hand, if the frequency of the controlling gene did not segregate to a normal distribution, the phenotype would be a qualitative character (Allard 2005). The result of segregation analysis on the Tanggamus X Taichung cross at $F_{2:3}$ generation progeny fitted the frequency of normal distribution for pod number- per plant and seed weight per plant thus, these characters were quantitatively controlled by many minor genes having small influence of each (Crowder 1997). The expression and extent of a quantitative character were dependent on environmental factors (Baihaki 2000). The result was in accordance with the results of Sriwidarti (2010) and Wulandari (2013) which concluded that pod number and seed weight per plant on soybean and peanut followed a normal distribution with a peak and therefore were controlled by many genes.

The segregation ratio of plant height was 3: 1 indicating that the character was controlled by a dominant gene having 25 – 50 % of augmentation (Table 3). The segregation ratio of 100-seed weight per plant was 13: 3 indicating for a dominant gene with only 10 – 25 % of augmentation (Table 4). Therefore, the 100 seed weight per plant character was regulated by two genes that interacted as dominant-recessive epistasis; dominant gene at one locus and the recessive gene on another locus effecting the appearance of the same phenotype (Crowder 1997).

Table 3. Chi-squared segregation ratios for plant height on the Tanggamus X Taichung crossed population at $F_{2:3}$ generation

Ratio	Observation	Expectation	χ^2count	$\chi^2\alpha_{0.01}$	P $\chi^2>\alpha_{0.01}$
Two Classes					
3:1	67 : 27	70.5 : 23.5	0.60		0.25-0.50
9:7	67 : 27	52.88 : 41.12	8.71**	6.64	<0.005
13:3	67 : 27	76.38 : 17.63	5.75		0.01-0.05
15:1	67 : 27	88.12 : 5.88	77.71**		<0.005
Three Classes					
1:2:1	58 : 34 : 2	23.5 : 47 : 23.5	73.91**		<0.005
9:3:4	58 : 34 : 2	52.88 : 17.62 : 23.5	35.38**	9.21	<0.005
9:6:1	58 : 34 : 2	52.88 : 35.25 : 5.88	3.10		0.10-0.25
12:3:1	58 : 34 : 2	70.5 : 17.63 : 5.88	19.99**		<0.005
Four Classes					
9 : 3 : 3 : 1	58 : 30 : 5 : 1	5.88 : 17.63 : 17.63 : 52.88	22.27**	11.35	<0.005
6 : 3 : 3 : 4	58 : 30 : 5 : 1	35.25 : 17.63 : 17.63 : 23.5	53.96**		<0.005

Note: **= different at $\alpha_{0.01}$

Based on the results of the study the selection for the pod number and seed weight per plant could not be done in early generations. The characters were controlled quantitatively by many minor genes, or polygenic, where each gene contributes small to the appearance or expression of a certain quantitative character in an additive fashion (Baihaki 2000). Similarly, 100 seed per plant character was not effective to be used on early generation selection since the character was controlled by a dominant-recessive gene epistasis. The results also showed that the gene action in controlling disease severity included a dominant gene action over positive and negative, as well as the most dominant positive and negative (Sa'diyah et al. 2016). Therefore, the selection for resistant characters would only be effective when done on further generations.

Table 4. Chi-squared segregation ratios for the 100 seed weight per plant on the Tanggamus X Taichung crossed population at the $F_{2:3}$ generation

Ratio	Observation	Expectation	χ^2count	$\chi^2\alpha_{0.01}$	P $\chi^2>\alpha_{0.01}$
Two Classes					
3:1	81 : 31	70.5 : 23.5	6.56		0.05-0.01
9:7	81 : 31	52.87 : 41.12	34.36**	6.64	<0.005
13:3	81 : 31	76.38 : 17.63	1.71		0.25-0.10
15:1	81 : 31	88.12 : 5.87	8.13**		<0.005
Three Classes					
1:2:1	58 : 34 : 2	23.5 : 47 : 23.5	73.91**		<0.005
9:3:4	58 : 34 : 2	52.87 : 17.63 : 23.5	35.38**	9.21	<0.005
9:6:1	58 : 34 : 2	52.87 : 35.25 : 5.8	3.10		0.25-0.10
12:3:1	58 : 34 : 2	70.5 : 17.63 : 5.87	19.99**		<0.005
Four Classes					
9 : 3 : 3 : 1	58 : 05 : 30 : 1	52.87 : 17.63 :17.63 : 5.87	531.10**	11.35	<0.005
6 : 3 : 3 : 4	58 : 05 : 30 : 1	35.25 : 17.63 : 17.63 : 23.5	53.96**		<0.005

Note: **= different at $\alpha_{0.01}$

The segregation of characters resistant to SMV evaluated on the $F_{2:3}$ generation of Tanggamus X Taichung progenies was expected to facilitate for the selection method in the next generation. The results of the study indicated that the selection would not be effective in early generation $F_{2:3}$ because the heterozygosity level was high. Selection would be more effective when done in later generations when the level of heterozygosity was greatly reduced to increase homozygosity to a higher level. The study showed three $F_{2:3}$ genotypes which retained the resistance to the SMV infection as well as yielded greater than both parents, Tanggamus and Taichung (Table 5).

Table 5. The genotype selection based on of pod number per plant, seed weight plant plant and disease severity characters as expected from Tanggamus X Taichung crossed population at $F_{2:3}$ generation

No.	Genotype No.	Pod Number per plant	Seed Weight per plant (g)	Disease Severity (%)	Class Criteria of Disease Severity
1	6.6.22	126	21.28	25.00	Tolerant
2	6.6.24	136	31.03	25.00	Tolerant
3	6.6.65	86	16.05	25.00	Tolerant
Average $F_{2:3}$		77	13.40	32.81	Partial Tolerant
Average $F_{2:3}$ Selected		116	22.79	25.00	Tolerant
Average Tanggamus		42.17	5.55	28.27	Partial Tolerant
Average Taichung		69.67	13.76	33.61	Partial Tolerant

Note: Class criteria of Disease Severity were Highly Tolerant (0 – 15 %); Tolerant (16 – 25 %); Partial Tolerant (26 – 35 %); Partial Susceptible (36 – 55%); Susceptible (56 – 75 %); and Highly Susceptible (76 – 100 %), respectively.

CONCLUSIONS

The segregation patterns of soybean resistance to SMV infections of the progenies of Tanggamus X Taichung $F_{2:3}$ generation were in accordance to the Mendelian ratio or its modification of 1: 2: 1, plant height of 3: 1, and 100 seed weight per plant of 13: 3. There were three genotypes resistant to SMV infection and yielded greater than the both parent, Tanggamus and Taichung. The $F_{2:3}$ generation of Tanggamus X Taichung crossed progenies showed pod number per plant and seed weight per plant characters distributed normally. The resistance to SMV infections segregated following Mendelian modification of 1: 2: 1 indicating that the resistance was controlled by a partially dominant single gene. Therefore, it would not be complicated to develop soybean lines resistant to SMV.

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