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Full Length Article

Resistance to Soybean Mosaic Virus with High Yield on F7 Soybean Lines

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Abstract

Soybean mosaic virus (SMV) is one of the viruses, which can reduce the quality and the amount of soybean [*Glycine max* (L.) Merr.] production. Control of the disease by planting resistant varieties to SMV is considered as the best method and environmentally safe. The aim of this research was to identify resistance to SMV with high yield on F7 soybean lines. Each of 56 best F7 lines was obtained from selection of F2 to F6 lines using the modified bulk method. Every breeding line was planted together with its parents in one block. Each line was grown in a 2 m × 3 m plot at a 40 cm × 15 cm plant spacing. The trial was set in a randomized complete block design with three replications. Resistance to SMV-T isolate was analyzed by symptom observation and serological detection using Double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA). The symptoms varied from mild, mosaic, necrotic and symptomless reactions. Out of the 56 soybean lines, 54 soybean lines reacted negative. Titre of virus showed absorbance values lower than two lines were susceptible, i.e. (1) W/PI 200485-7-8; (2) GK/Mlg 3288-7-11. The highest yield (2.59 t/ha) with the lowest disease severity category achieved by lines (GK/PI 200485-7-8). There were significant differences in disease severity and seed yields between Wilis and Gepak Kuning varieties as parents with high yield potential in breeding line. The path analysis indicated that the number of pods per plant directly affected the seed yields.

Keywords: *Glycine max* L.; Resistance; DAS-ELISA; SMV-T isolate; Seed yields

Introduction

This time, Indonesia is preparing Feed Indonesia-Feed the world Program for sustainability of national food security. Soybean is one of the strategic food commodities whose demand is increasing. Indonesian government conducted some programs to increase domestic production such as application of high yielding varieties. Superior variety of soybean early maturing, large seed size, and high potential still become priority of consumer, but is not done for resistance to Soybean mosaic virus (SMV).

In East Java province, yield reduction 15−35% have been observed in SMV susceptible soybean. Mosaic disease is one of the obstacles adoption soybean varieties (Andayanie *et al.*, 2011). The availability of improved varieties of resistance to SMV and high yield are needed for increasing the domestic soybean production. Therefore, several efforts had been done to increase the soybean production that were still constraints in their implementation, namely the virus infection could not be handled accordingly yet.

SMV is a member of the genus Potyvirus, a major disease of soybean and has currently become one of the main constraints of soybean production in the world. Symptom of infection include mosaic and necrosis (Zheng *et al*., 2006). Furthermore, an isolate of SMV isolate T from soybean fields in Ngawi, East Java, Indonesia has been isolated and characterized symptomatologically and serologically as well as molecularly. On Soybean varieties such as; Wilis, Gepak Kuning, this virus causes stunting of plant, crinkling of leaves, decrease in seed quality (mottle and deformation of seeds) (Andayanie and Adinurani, 2013). Isolates of SMV that cause different symptom on soybean. Symptoms vary with host genotype, virus strain, environment, plant age at infection, although the symptoms cannot be used as reliable criteria. SMV is a seed borne viral pathogen and aphids can efficiently spread it from plant to plant, it is difficult to control the virus. The virus could easily be transmitted by the soybean seeds and insect vector (*Aphis glycines*). SMV infection through seeds of infected plants, but the infectivity of the SMV through mottled and non-mottled seeds of soybean Wilis varieties

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was not known yet (Andayanie, 2012). The mosaic disease incidence usually increases during the dry seasons along with the increases of the insect vector populations. Changes in insect vector, populations over the last several years have increased the incidence of SMV. So far, there is no effective chemical to control this virus disease. Therefore, resistance to SMV must be improved and incorporated into selected lines to minimize yield loss.

SMV is mainly transmitted by *Aphis glycines* and spread from cell to cell rapidly. However, control of the aphid vector has not proved reliable as means of resistance varieties. Resistance breeding is one of the solution to overcome the disease with low yield. Hybridization between different genotypes aims to obtain descents, which inherits with the good characters of both plants. Therefore, soybean genotypes resistant to SMV are needed as parents in soybean breeding program for resistance to the disease. Soybean genotypes resistant to SMV isolate T with low yield found in the collection of soybean of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor-Indonesia throughout selection and evaluation. To face the SMV, these soybean genotypes and varieties are the valuable resource for breeding program. The genetic variability is highly desirable for developing new cultivars. The Soybean genotypes such as; L. Jombang, Mlg 3288, L. Temanggung, Malabar, Pangrango, PI 200 485, M8 Grb 44) were resistant to SMV isolate T while Wilis and Gepak Kuning varieties were susceptible with high yield (Andayanie and Adinurani, 2014; Andayanie and Sulistyo, 2015). The genetic variability is highly desirable for developing new cultivars, which is selected by soybean lines resistance to SMV and seed yield.

Genetic improvement of resistance to SMV with high yield is an approach that is inexpensive and easy to be implemented. New varieties of soybean has important and strategic role in efforts to increase production. Planting resistant varieties could warrant yield stability under the changing of environmental condition (Suyamto, 2014). Control of the disease by planting resistant cultivars to SMV is considered as the best method and environmentally safe. However, soybean varieties resistance to SMV are currently not available. Therefore, the objective of this research was to identify resistance to SMV with high yielding on F7 soybean lines. If this hypothesis is correct, then this resistance it should be incorporated to soybean lines for further advances in a new variety of soybean.

Materials and Methods

The Genetic Material

Soybean genotypes resistance to SMV i.e., L. Jombang, Mlg 3288, L. Temanggung, Malabar, Pangrango, PI 200485, M8Grb 44 were used as parents in the cross combination as a donor of resistance to SMV. L. Jombang, Mlg 3288, L. Temanggung, Malabar, Pangrango, PI 200485, M8Grb 44 are soybean germplasm collection from Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD). Wilis and Gepak Kuning varieties are susceptible to SMV-T isolate and *Aphis glycines* (Andayanie and Adinurani, 2013; Center for Food Crops Research and Development, 2010). Wilis and Gepak Kuning varieties are used as parents with high yield potential in the cross combination.

Selection of F5, F6 and F7 soybean breeding lines was conducted in the soybean field of Jenggrik Village, Kedunggalar Subdistrict, Ngawi District, Indonesia. In season II the 140 F6 (4−8 lines/population) breeding lines which were selected from the F5 lines in season I and then as many as 56 were selected resistant to SMV and high yield. Those 56 soybean lines were grown in season III. Fifty six F7 soybean lines of seven germplasms and two varieties were evaluated resistant to SMV with high yield. Every breeding line was planted together with its parents in one block. Bulk modification method based selection was used in F2−F7 generation (modified by Yacub *et al*. 2013). Selection procedure was done in each generation. Each line was grown in a 2 m × 3 m plot at a 40 cm × 15 cm plant spacing. The trial was set up in a randomized complete block design with three replications. Susceptible varieties are planted in 10 rows of test plants.

Maintenance of Virus Source, *Aphis glycines* and Inoculation Procedures

The SMV-T isolate inoculum was preserved from Laboratory of Plant Virology, Department of Agrotechnology, Faculty of Agriculture, Merdeka University, Madiun and propagated on soybean (*Glycine max* L.) in a whitefly-proof screen house, at the Merdeka Madiun University. SMV-T isolate caused necrotic symptoms in susceptible soybean. Inoculation was done on surface leaves of soybean 10 days old, completely-expanded seedling were lightly dusted with carborundum (400 Mesh) and rub-inoculated with virus-infected sap (1:10 dilution leaf material:buffer) using sponge plugs and grown for 21 days. Inoculation of SMV-T isolate were used to detect resistance gene in F1 to F6. Susceptible varieties were mechanically inoculated with SMV-T isolate week after planting.

SMV is non-persistently transmitted, brief probing by *Aphis glycines* is sufficient for successful transmission (Wang and Ghabrial, 2002). Adult *Aphis glycines* were obtained from soybean plants in the village Jenggrik, District Kedunggalar, Ngawi and identified using the identification key of Martin (1987). The insect vector (*Aphis glycines*) were reared on soybean plants in whitefly-proof cages. They were starved for 1.5 to 2 h, then placed on soybean leaves infected with isolate of SMV-T isolate. The insect were given access to SMV-T isolate infected soybean plants.

After 5–10 min acquisition access period, the insect vector were re-collected individually using an aspirator. Five to ten aphids were transferred to F7 soybean lines (3 to 5 plants per lines), allowed to feed for 24 h, then were sprayed with Nissuron 50 EC. Symptoms on inoculated plants were recorded during the following 4 weeks. The presence of SMV-T isolate in the test plants was determined by ELISA (Enzyme-linked immunosorbent assay).

Serological Detection

Serological detection was used to determine concentration of virus in soybean lines, which were inoculated with SMV-T isolate then tested using DAS ELISA (double antibody sandwich-enzyme linked immunosorbent assay). DAS ELISA has proved to be a sensitive and rapid test for detection of soybean viruses in a large number of soybean samples and thus are commonly used for SMV detection. DAS ELISA was performed, using a polyclonal antiserum against SMV according to the Clark and Adam (l977). Positive (supplied by DSMZ, AS-05431) and negative (healthy soybean) control were included on each micro titer plate. Microtiter plate covered SMV antibodies (dilution with buffered phosphate 1:100) and incubated for 14-16 h at 4°C, then washed wells with Phosphate buffered saline tween (PBST) four times. Samples (0.1 g) were mixed with 2 mL buffer carbonate until powder then was vortexed and filtered with sterile cotton. The supernatant poured well micro titer plate with micro pipette each 100 µL. The empty well was filled with sterile water and incubated for 14-16 h at 4°C. The sap was removed and microtiter plate was washed with PBST four times then incubated with conjugate (ratio buffered phosphate 1:100) each well 100 µL for 16 h at 4°C. Conjugate removed and micro titer plate washed with PBST 4 times. Substrate buffer (100 µL) was filled at each wells and incubated for 45 min. at 4°C. Micro titer plate entered to ELISA reader (405 nm) to obtain absorbance value of ELISA test. Samples with absorbance values greater than or equal to three times of negative samples were considered infected (positive). ELISA absorbance value can used to confirm the disease severity.

The Disease Severity

Disease progression was surveyed in 10 randomly selected plants from each lines. Following Balogun and Bakare (2007), plants were scored for disease severity according to the scale of SMV as shown in Table 1.

Disease severity index (DS) was calculated for each population with the formula of Campbell and Madden (1990):

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

DS = The disease severity index (%)

n = Sum of infected plants in each category

V = Value scores of each category

N = Total number of plants assessed

Table 1: The scales of *Soybean Mosaic Virus* (SMV) symptom

Scale	Symptom	Reaction of plant
0–15%	Leaf healthy	Very tolerant
16–30%	Mosaic symptom	Moderately tolerant
31–50%	Mosaic symptom with small leaf	Mildly tolerant
51–80%	Mosaic symptom with small leaf and curly	Susceptible
81–100%	Mosaic symptom with small leaf, curly, and stunting	Very susceptible

Z = Maximum category (4).

Plants showing indistinct or no symptom at the final observation were assayed by back inoculation to *Chenopodium amaranticolor*.

Statistical Analysis

Percent disease severity, ELISA absorbance reading, and seed yield were subjected to analysis of variance. Percentage values were arc sign transformed before analysis. The treatment means were compared using fisher's least significant difference (LSD) test at $P = 0.05$ (Steel and Torrie, 1980). Effectiveness of varieties as parents with high yield potential, i.e. Gepak Kuning and Wilis were compared using the t test. Direct and indirect effect of seed yield on F7 soybean lines were identified using the path analysis.

Results

Symptom Development of F7 Soybean Lines by SMV-T Isolate

The infected soybean plants started to develop the SMV symptom after 21 days. In Gepak Kuning and Wilis varieties, infection with SMV manifested as leaf mosaic, which progressed to leaf wrinkling. Based on symptom development that F7 soybean lines showed no symptom and characteristic mosaic after insect transmission with SMV-T isolate. Within 21 to 24 days post transmission, W/PI 200.485-7-8 and GK/Mlg 3288-7-11 lines displayed mosaic symptom on emerging trifoliate leaves. There was variation in the severity of mosaic symptoms depending upon soybean lines. The development symptoms showed systemic and appeared as light and dark green patches on individual leaves. Leaf blades are often narrow with dark green swollen veins and become puckered along the veins and curled upward.

Serological Detection

DAS ELISA method was used to detect SMV-T isolate in F7 soybean lines, check varieties and genotypes. The serological detection based on the reaction between antigens to antibody. Antigenic binding site on antibody protein would match antigenic determinant site from protein-

Table 2: Reaction to SMV isolate T and seed yield of selected F7 soybean lines[a]

No.	Breeding line [b)]	DS [c)] (%)	SC [d)]	AEV [e)]	Reaction [f)]	SY [g)] (t/ha)
1.	W/L. Jombang-7-3	6.59 bc	0	0.27 ± 0.06 ef	R	1.61 ab
2.	W/L. Jombang-7- 9	10.83 ef	0	0.36 ± 0.08 fg	R	1.57 ab
3.	W/L. Jombang-7-12	14.47 ef	0	0.29 ± 0.09 ef	R	1.78 b
4.	W/L. Jombang -7-21	9.81 de	0	0.29 ± 0.11 ef	R	1.77 b
5.	W/Mlg 3288-7-1	14.52 ef	0	0.23 ± 0.12 de	R	1.65 ab
6.	W/Mlg 3288-7-5	11.68 ef	0	0.26 ± 0.03 de	R	1.83 b
7.	W/Mlg 3288-7-9	8.69 cd	0	0.27 ± 0.11 ef	R	1.79 b
8.	W/Mlg 3288-7-14	12.78 ef	0	0.34 ± 0.05 fg	R	1.59 ab
9.	W/L. Temanggung-7-1	9.72 de	0	0.21 ± 0.12 de	R	1.81 b
10.	W/L.Temanggung-7-9	4.75 ab	0	0.23 ± 0.10 de	R	1.61 ab
11.	W/L. Temanggung-7-12	13.87 ef	0	0.35 ± 0.11 fg	R	1.53 ab
12.	W/L.Temanggung-7-14	5.66 bc	0	0.11 ± 0.01 bc	R	2.32 d
13.	W/Malabar-7-3	14.72 ef	0	0.34 ± 0.02 fg	R	1.68 ab
14.	W/Malabar-7-7	7.58 cd	0	0.37 ± 0.05 fg	R	1.57 ab
15.	W/Malabar-7-9	14.49 ef	0	0.24 ± 0.09 de	R	1.66 ab
16.	W/Malabar-7-13	13.85 ef	0	0.36 ± 0.16 fg	R	1.56 ab
17.	W/Pangrango-7-1	9.74 cd	0	0.27 ± 0.06 ef	R	1.63 ab
18.	W/Pangrango-7-6	12.76 ef	0	0.39 ± 0.13 fg	R	1.55 ab
19.	W/Pangrango-7-12	10.78 ef	0	0.33 ± 0.14 fg	R	1.59 ab
20.	W/Pangrango-7-14	11.66 ef	0	0.32 ± 0.17 fg	R	1.55 ab
21.	W/PI 200.485-7-4	5.71 bc	0	0.22 ± 0.09 de	R	1.62 ab
22.	W/PI 200.485-7-8	30.68 g	1	0.61 ± 0.11 g	S	1.40 a
23.	W/PI 200.485-7-14	13.86 ef	0	0.20 ± 0.12 cd	R	1.50 ab
24.	W/PI 200.485-7-16	12.75 ef	0	0.33 ± 0.16 fg	R	1.58 ab
25.	W/M8Grb 44-7-2	17.62 ef	0	0.25 ± 0.07 de	R	1.61 b
26.	W/M8Grb 44-7-11	13.90 ef	0	0.37 ± 0.11 fg	R	1.59 ab
27.	W/M8Grb 44-7-15	5.67 bc	0	0.29 ± 0.14 cd	R	1.65 ab
28.	W/M8Grb 44-7-18	13.90 ef	0	0.41 ± 0.12 fg	R	1.53 ab
29.	GK/L. Jombang -7-6	11.68 ef	0	0.39 ± 0.15 fg	R	1.59 b
30.	GK/L. Jombang-7-10	8.65 cd	0	0.35 ± 0.10 fg	R	1.64 ab
31.	GK/L.Jombang-7-14	8.73 cd	0	0.39 ± 0.08 fg	R	1.66 ab
32.	GK/L.Jombang 7-18	4.78 ab	0	0.36 ± 0.18 fg	R	1.67 ab
33.	GK/Mlg 3288-7-3	10.76 ef	0	0.37 ± 0.19 fg	R	1.65 ab
34.	GK/Mlg 3288-7-6	9.82 de	0	0.39 ± 0.06 fg	R	1.71 b
35.	GK/Mlg 3288-7-11	32.35 g	1	0.59 ± 0.09 g	S	1.47 a
36.	GK/Mlg 3288-7-16	11.65 ef	0	0.25 ± 0.01 de	R	1.64 ab
37.	GK/L. Temanggung-7-6	13.91 ef	0	0.31 ± 0.06 fg	R	1.69 ab
38.	GK/L. Temanggung-7-9	8.71 cd	0	0.15 ± 0.03 cd	R	1.78 ab
39.	GK/L.Temanggung-7-16	14.50 ef	0	0.38 ± 0.01 fg	R	1.58 ab
40.	GK/LTemanggung-7-18	4.37 a	0	0.08 ± 0.03 a	R	2.48 d
41.	GK/Malabar-7-4	6.63 bc	0	0.22 ± 0.04 de	R	1.75 b
42.	GK/Malabar-7-8	7.58 cd	0	0.30 ± 0.15 fg	R	1.63 ab
43.	GK/Malabar-7-14	11.65 ef	0	0.35 ± 0.17 fg	R	1.66 ab
44.	GK/Malabar-7-17	12.78 ef	0	0.35 ± 0.10 fg	R	1.53 ab
45.	GK/Pangrango-7-13	7.61 cd	0	0.26 ± 0.13 de	R	1.71 b
46.	GK/Pangrango-7-9	5.68 bc	0	0.11 ± 0.05 bc	R	2.34 d
47.	GK/Pangrango-7-11	14.45 ef	0	0.38 ± 0.10 fg	R	1.68 ab
48.	GK/Pangrango-7-18	6.64 bc	0	0.21 ± 0.09 de	R	1.94 c
49.	GK/PI200.485-7-2	4.76 ab	0	0.18 ± 0.05 bc	R	1.81 b
50.	GK/PI200.485-7-8	5.72 bc	0	0.25 ± 0.07 de	R	1.73 b
51.	GK/PI 200.485-7-12	2.10 a	0	0.08 ± 0.04 a	R	2.59 d
52.	GK/PI200.485-7-17	2.14 a	0	0.09 ± 0.05 a	R	2.55 d
53.	GK/M8Grb 44-7-1	7.56 cd	0	0.19 ± 0.08 cd	R	1.95 c
54.	GK/M8Grb44-7-6	13.87 ef	0	0.29 ± 0.02 ef	R	1.56 ab
55.	GK/M8Grb44-7-8	5.68 bc	0	0.28 ± 0.15 ef	R	1.62 ab
56.	GK/M8Grb44-7-14	12.80 ef	0	0.32 ± 0.02 fg	R	1.59 ab
57.	Wilis	33.84 g	2	0.68 ± 0.08 g	S	1.34 a
58.	Gepak Kuning	31.52 g	2	0.63 ± 0.11 g	S	1.67 ab
59.	L. Jombang	11.72 ef	0	0.43 ± 0.01 fg	R	1.46 a
60.	Mlg 3288	9.86 de	0	0.38 ± 0.12 fg	R	1.61 ab
61.	L.Temanggung	10.81 ef	0	0.33 ± 0.17 fg	R	1.48 a
62.	Malabar	13.89 ef	0	0.46 ± 0.02 fg	R	1.43 a
63.	Pangrango	8.70 cd	0	0.39 ± 0.01 fg	R	1.52 a
64.	PI 200.485	6.56 bc	0	0.23 ± 0.04 de	R	1.65 ab
65.	M8Grb 44	11.74 ef	0	0.45 ± 0.07 fg	R	1.55 ab
HC [h)]				0.19 ± 0.02		
PC [i)]				0.57 ± 0.08		

[a]Mean ± standart deviation. Values sharing same letters differ non significantly (P > 0.05) [b]Breeding lines of W code was from Wilis; breeding lines of GK code was from Gepak Kuning

[c]DS = disease severity; [d]SC = symptom score (0 = very tolerant, 1 = moderately tolerant, 2 = midly tolerant, 3 = susceptible, 4 = very susceptible); [e]AEV = Absorbance ELISA Value; [f]R = resistant (≤ 0.57); S = (> 0.57); [g]SY = seed yield (t/ha); [h]HC = healthy control (negative control); [i]PC = positive control

containing antigenic. Antibody of virus have to match and not caused with antibody from the another virus (Clark and Adams, 1977). Samples of newly developed leaves of the infected soybean plants were tested using

Table 3: Comparison of 10 selected lines from both susceptible to SMV varieties

No.	Breeding line	SY[a] (t/ha)	DS[b] (%)	Breeding line	SY[a] (t/ha)	DS[b] (%)
1.	W/L. Temanggung-7-14	2.32	5.66	GK/PI200.485-7-12	2.59	2.10
2.	W/Mlg 3288-7-5	1.83	11.68	GK/PI200.485-7-17	2.55	2.14
3.	W/L. Temanggung-7-1	1.81	9.72	GK/L.Temanggung-7-18	2.48	4.37
4.	W/Mlg 3288-7-9	1.79	8.69	GK/Pangrango-7-9	2.34	5.68
5.	W/L. Jombang-7-12	1.78	14.47	GK/M8Grb44-7-1	1.95	7.56
6.	W/L. Jombang-7-21	1.77	9.81	GK/Pangrango-7-18	1.94	6.64
7.	W/Malabar-7-3	1.68	14.72	GK/PI 200.485-7-13	1.81	4.76
8.	W/Mlg 3288-7-1	1.66	14.49	GK/L.Temanggung-7-9	1.78	8.71
9.	W/Mlg 3288-7-1	1.65	14.52	GK/Malabar-7-4	1.75	6.63
10.	W/PI 200.485-7-4	1.63	9.74	GK/PI200.485-7-8	1.73	5.72
t values (seed yield/ha)	2.53*					

Asterisks indicate a significant difference between two varieties based on the t test (P= 0.05)
[a]SY = seed yield; [b]DS = disease severity

Table 4: Direct effect (in diagonal) and indirect effect of seed yield on F7 soybean lines

Character	PH	MA	BP	PP	MS	DS	r_{xy}
PH	-0.0413	0.3184	0.0000	0.3975	-0.1582	-0.0095	0.9044
MA	-0.0162	0.7498	0.0000	0.4603	-0.2147	-0.0465	0.9327
BP	-0.0149	0.3010	0.0000	0.4102	-0.1365	-0.0352	0.5246
PP	-0.0191	0.3897	0.0000	0.7505	-0.2319	-0.0542	0.8350
MS	0.0164	-0.3593	0.0000	-0.4945	0.2568	0.0452	-0.5354
DS	0.0398	-0.2985	0.0000	-0.4085	0.1145	0.1165	-0.4362

PH = Plant height (cm); MA = Maturing age (days); PP = Number of seeded pods per plants; MS = Mass of 100 seeds (g); BP = Number of branches per plants; DS = Disease severity; r_{xy} = Total effect

ELISA kit. Based on results, reaction of 56 soybean lines to SMV isolate T and seed yield are given in Table 2.

Seed Yield

Analysis of variances showed that there were significant differences among the breeding lines tested against seed yield. The seed yield of GK/PI200.485-7-12 line was the highest (2.59 t/ha), not significantly higher than GK/PI200.485-7-17 line (2.55 t/ha). The range of seed yield of the 56 soybean lines tested range from 1.40 to 2.59 t/ha, while the parent check varieties and genotypes have ranged from 1.34 to 1.77 t/ha. GK/PI 200.485-7-12, GK/PI 200.485-7-17, and GK/L. Temanggung-7-18 lines were inoculated with SMV-T isolate, all 45 plants developed symptomless reaction. On the other hand, there were no significant differences in absorbance value, disease severity and seed yield (P> 0.05).

In breeding program hybridization provides unlimited possibilities of generating new combination character, which can be selected in the segregating lines. Comparison of 10 selected lines produced high yield and low SMV disease severity from both susceptible to SMV varieties using the t test showed that Gepak Kuning varieties were better than Wilis varieties.

Comparison of 10 selected lines resistant to SMV with high yield using the t test showed that Gepak Kuning variety as susceptible parents with high yield potential was better than Wilis variety. The disease severity differed ranging between 2.10−14.72%. The highest yield (2.59 t/ha) with low disease severity category also achieved by lines (GK/PI 200.485-7-8). The data also showed that there were significant differences in disease severity and seed yields (Table 3).

Result of path analysis indicated that the number of seeded pods per plant (PP) gave a direct effect on the seed yield. The number of branches per plant (BP) did not affect on the seed yield directly, but it affected the yield indirectly on the number of seeded pods per plant (Table 4).

Discussion

Plants were classified resistance if a symptomless reaction occurred, and susceptible if mosaic or necrotic symptoms occurred. No local lesion developed on *C. amaranticolor* leaves when inoculated with inoculum prepared from symptomless plants of the resistant soybean lines that were inoculated with SMV-T isolate (data not shown). It was confirmed to be present by DAS-ELISA values.

The dissemination would depend upon inhibition of virus particle replication in the leaf tissue of resistant plants. Resistance involve infective virus particle and restriction of local cell-to-cell movement along with reduced movement into and from the vascular system. Furthermore, the resistance conditioned by genes was non-necrotic and non-strain of virus specific. Virus resistance genes of those lines that function by restricting virus movement. According to Andayanie and Adinurani (2013) L. Jombang, Mlg 3288, L. Temanggung, Malabar, Pangrango, PI 200.485, M8Grb 44 genotype were found to possess a high degree of resistance to SMV-T isolate. There was no maternal effect on genetic inheritance for resistance

to SMV. The resistance in L. Jombang, Mlg 3288, M8Grb 44 were controlled by two recessive genes located at difference locus with a duplicate recessive epistatic interaction. The resistance in PI 200.485 and L. Temanggung were controlled by a single dominant gene, while that in Malabar and Pangrango were controlled by two dominant genes located in at different locus with a duplicate recessive epistatic interaction. The heritability number indicating that genetic factors played more important role in governing the resistance of soybean to SMV. Resistance to SMV strains that produce mosaic symptoms was shown to be conditioned by single dominant gene, whereas resistance to a severe isolate (necrotic symptoms) was shown to be conditioned by a single recessive gene (Lim, 1985).

Detection of virus depends on the concentration of virus in samples. Out of the 56 soybean lines, 54 soybean lines reacted negative (resistant reaction). These symptomless plants were infected with a titre of virus below detection threshold of ELISA. On the other hand, titre of virus showed absorbance values lower than two lines were susceptible. Lines reduced significantly the concentration of SMV-T isolate in plants as proved by a significant (P= 0.05) decrease in the absorbance value of ELISA reaction. The anti-SMV antibodies gave positive reaction with extracts from infected plants as shown by development of a obvious yellow color in ELISA miroplate wells. Soybean lines were infected with SMV isolate T (susceptible reaction) by DAS ELISA, i.e. (1) W/PI 2004.85-7-8; (2) GK/Mlg 3288-7-11. Absorbance values for samples of 2 healthy control (negative control). In the former virus content was consistenly high and all the leaves were ELISA positive. Even in those that were positive virus titre was much lower compared to the lower leaves of the susceptible varieties. SMV-T isolate produced mosaic symptom with small leaf on these lines. Fifty four soybean lines showed resistance to SMV after aphid transmission on susceptible varieties during the course of the 21 days experiment and no virus was detected by DAS ELISA whereas uninfected samples remained colorless in ELISA microplate wells. Among these lines, GK/L.Temanggung-7-18 showed minimum A 405 $_{nm}$ values (0.08 ± 0.03) followed by GK/PI 200.485-7-12 (0.08 ± 0.04), respectively. Resistance alleles in lines were probably conditioned by the same gene, since reactions of lines to SMV-T isolate and seed yield were similar.

Resistance to all seven SMV strains transferred from PI 360.844 to the soybean line OX 670 was shown to be conditioned by a dominant gene *Rsv2*. Cross combination technique used for crop improvement over the past few decades has shown that it is an effective breeding method to improved yield, and resistance to SMV in soybean (Cho and Goodman, 1979; Lim, 1985). According to Koo *et al*. (2005) resistance genes (*Rsv*1, *Rsv3* and *Rsv4*), have been genetically identified and deployed in United States germplasm of soybean for disease control. However, emergence of resistance breaking strains of SMV has been documented in other regions of the world.

The actual prevalence and incidence of SMV underestimated when based upon visual assessment for symptoms. Serological assay results revealed a variation in virus incidence among soybean lines. The resistance could involve an inhibition of virus particle replication in the tissue of resistant host plants and transformation of the virus in the plant. Consequently, it decreases virus distribution in plant organ. The selected line of resistance to SMV with high yield was evaluated for its symptom, disease severity and yield. Value severity of the disease in each lines was grew along with the resistance of plants with the same level of severity disease. These trait of a soybean are a result of the variety's genetic potential. This indicates that SMV isolate T mechanism depend upon the resistance genes. Selection based on the number of seeded pods per plant was used to the process of section F7 soybean lines for high yield character. According to Andayanie and Adinurani (2013), the heritability number indicating that genetic factors played more important role in governing the resistance of soybean to SMV.

Conclusion

Characters of agronomic importance can be used as selection of F7 soybean lines on yield component which have a large positive value with direct effect. Therefore, selection based on number of pods per plant is used to process of selection for high yield character.

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