

RESEARCH PAPER

Response of soybean lines to *Soybean mosaic virus* under drought stress

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ABSTRACT

The aim of this study was to assess soybean lines response to infection of *Soybean mosaic virus* (SMV) under drought stress. The experiment was conducted at the glasshouse in factorial in Randomized Complete Block Design (RCBD) with four replications. The first factor is soybean lines with four soybean lines (GK/PI, GK/M8Grb, W/M, GK/LT) including one susceptible check variety i.e. Anjasmoro. The second factor is drought stress with three levels of soil water content (100, 75, and 50%) field capacity. Seven days after planting (DAP), plants were inoculated with sap from leaves SMV infected soybean. The result showed that drought stress levels had affected the percentage of seed weight loss in GK/L-T than in the Anjasmoro variety. The number of leaves was slowly decreased from 42 to 49 DAP. The level, duration, and frequency of drought stress affected more significant in the inhibition of the seed filling phase. The GK/L-T reaction was not detected in the presence of SMV and also the lowest of Absorbance ELISA Value. The seed yield (t/ha) of GK/L-T that was most superior and the lowest percentage of disease severity under drought stress.

Key words: disease severity, dry season, *Glycine max* L. Merr, soil field capacity

INTRODUCTION

In Indonesia, about 70% of soybean (*Glycine max* L. Merr.) was planted on the paddy fields during the first dry season (February–June) after the first rice harvest season and during the second dry season (June–September) after the second rice harvest season. About 30% of soybean planting is performed on the dryland during the first dry season. However, production of dryland-soybean is facing some problems, mainly with the limited water availability as well as the low organic matter. Therefore, some or all of the plant growth phases experience drought stress. Plants have responses to improve water status by: 1) change the distribution of assimilate to support root growth, thereby increasing root capacity to absorb water, inhibiting leaf expansion and leaf number from reducing transpiration; 2) regulating stomatal opening to prevent water loss through transpiration (Fathi & Tari, 2016). Therefore, varieties, duration of drought stress, and growth stages determine yield loss on soybean plants due to water deficit. Moreover, *Soybean mosaic virus* (SMV) is one of the most serious viruses that can decrease soybean grain yield.

SMV showed symptoms of vein clearing, mosaic, and mottling on leaves (Andayanie et al., 2017). Low rainfall and high temperatures cause an explosion of insect vectors of the virus. Therefore, *Soybean mosaic virus* (SMV) and *Cowpea mild mottle virus* (CPMMV) are often infected (Andayanie & Ermawati, 2019; Andayanie et al., 2019a).

Plants will show different levels of tolerance to drought stress. The drought susceptibility index can show drought stress in the plant growth stages. During the vegetative period, the drought stress causes small leaves and small stem diameters of soybean and it will decrease the plant weight. The water shortage stresses in each growth period of soybean resulting in the decline of the yields. The most significant phases of the plant affecting the yield are in the flowering stage, seed formation, and pod filling period. Drought stress during the flowering stage causes increased shedding of the flower. Once the drought stress continues to the formation and pod filling stage, the yield will be significantly decreased. Drought stress in both growth stages also causes imperfection of pod filling, resulting in smaller soybean seeds and a reduction in dry seed weight (Liu et al., 2004; Li et al., 2018).

Virus infection can improve plant tolerance in drought stress which correlates with increased osmoprotectant and antioxidant levels in infected plants. In *Turnip mosaic virus* (TuMV), infection induces a change in the plant physiological homeostasis. Virus-infected plants showed slower drying symptoms

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than control treatments. This virus can promote host survival in situations of abiotic stress. However, the intensity and duration of drought stress cause decreased fitness or mortality for their host (Xu et al., 2008; González et al., 2021).

Soybean varieties, duration of drought stress, growth period as well as SMV infection significantly affected the yield loss on soybean plants. These problems can be resolved by developing soybean varieties which are tolerant to drought stress and SMV. This study was performed to evaluate the growth and yield of soybean (*Glycine max* L. Merr.) lines against drought stress and SMV.

MATERIALS AND METHODS

Research Site. The experiment was conducted in a greenhouse in Sidwayah District, Ngawi Regency (East Java Province- Indonesia). During the study, a temperature was in the range of 28.8 to 29.6 °C with a relative humidity in the range of 75 to 82% and an average rainfall of 103 mm per month with the number of rainy days was 13. The planting media was alfisol soil.

Plant Preparation and Maintenance. Soybean seeds were obtained from soybean breeding lines of F6 selection (Andayanie et al., 2017). The seeds were sown in polybags measuring 20 × 30 cm with two seeds per polybag. Organic and inorganic fertilizers equivalent to 100 kg SP36/ha (0.21 g/polybag, 50 kg urea/ha (0.105 g/polybag), and 5 kg KCl/ha (0.105 g/polybag) were applied at the time of planting by evenly mixed with the growing media. Urea and KCl fertilization were applied at 7 and 35 days after planting (DAP). Weed control was carried out using the botanical herbicide of cashew nut shell extract, as described by Andayanie et al. (2018).

Soybean mosaic virus Inoculation. SMV isolate T was obtained from susceptible soybean with mosaic symptoms in Ngawi District. The presence of the virus was confirmed using Indirect-enzyme-linked immunosorbent assay (ELISA) with the modified Koenig (1981) method. Samples of leaves with mosaic symptoms were crushed in an extraction buffer (0.05 M sodium carbonate buffer pH 9.6) until pulverized then filtered and taken sap as antigen (w/v=1:10). Polyclonal antibodies SMV are diluted 1000 times each from an initial titer of 1000–8000. A polystyrene microtiter plate (Nunc-immuno-Plate, InterMed) was added to each well with: (1) 100 µL of the antigen that and incubated for 4 hours at 37 °C; (2) 150 µL BSA 0.05% for

60 min at 37 °C; (3) 100 µL antibody for 18 hours at 40 °C; (4) 100 µL diluted general conjugate 3000 times then incubated for 2 hours at 37 °C; (5) 200 µL of the substrate in the form of 1 mg/mL p-nitrophenyl phosphate in 10% diethanolamine pH 9.8 for 1–1.5 hours at room temperature. At the end of the stage, the microplate was washed three times with phosphate buffer saline tween 20 (PBS-T) 0.05%. The test result was seen by measuring the absorbance value at a wavelength of 405 nm using an ELISA Reader (Allsheng Model FlexA-200, Zhejiang, China).

Field Capacity Determination. The field capacity of the planting media is determined based on the amount of water that fills the soil pores (Ghorbani et al., 2017). The water content was calculated as follows:

$$W_c = \frac{W_w - O_{dw}}{O_{dw}} \times 100\%$$

W_c = the water content is added to reach field capacity;

W_w = wet weight of the soil field capacity;

O_{dw} = oven-dry weight.

The wet weight of the soil (W_w) was determined by watering the growing media until it was saturated, which was indicated by the presence of water dripping from the growing media. The surface of the planting media was covered using plastic transparent to avoid evaporation and left for 48 hours until there was no more dripping water. Finally, 100 g of the soil was taken as the wet weight of soil field capacity (W_w) data. The sampling methods used a composite sample with three replications. The dry weight of the soil (O_{dw}) was determined by incubating the wet weight of the soil field capacity (W_w) of the growing medium at 110 °C for 48 hours. Then, the soil samples were put in a desiccator at room temperature and weighed to obtain the oven-dry weight (O_{dw}) data. The water was added in growing media to adjust to drought stress treatments. The water was added according to the initial volume before planting.

Screening of the Soybean Varieties Tolerant to SMV.

The observed plant samples were selected from each line. Disease severity was recorded using the scale of SMV infection described by Andayanie et al. (2017) (Table 1).

The disease severity was calculated using the formula:

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

- DS = the disease severity (%);
- n = the sum of infected leaves in each category;
- v = value score of each category;
- N = total number of observed leaves per plant;
- V = the highest category.

Data Analysis. The experiment was performed in a two factor factorial experiment under Randomized Completely Block Design (RCBD) with four replicates. Ten plant samples were used in each treatment. The first factor was soybean lines. Four soybean lines and one variety were used, i.e GK/PI, GK/M8Grb, W/M, GK/L-T and one susceptible check variety (Anjasmoro). The second factor was drought stress, consisting of three levels of soil water content (100, 75 and 50% of field capacity). Drought stress treatment was carried out 14 DAP. Observations were started at 21 DAP on plant height, number of leaves and seed weight, 100 seeds weight, response of the soybean lines to SMV under drought stress, the disease severity, seed yield on soybean lines under drought stress at generative period. Data were analyzed using analysis of variance (ANOVA), followed by Tukey’s test using Minitab software 17.3.0. (Abdi & Williams, 2010).

RESULTS AND DISCUSSION

The Plant Height. The level of drought stress had a significant effect ($P < 0.05$) on the plant height at 21 to 49 DAP. The soybean lines differently responded to

drought stress and showed significant differences ($P < 0.001$) in the plant height. Pour-Aboughadareh et al. (2020) reported that decreased water availability affects plant height. Pressure on cell turgor affects the ability of cells to elongate and enlarge, resulting in delayed plant growth and development (Andayanie et al., 2019b). The average plant height of check variety (Anjasmoro) was not significantly different from GK/PI and GK/M8GRB and GK/L-T but it was significantly different from other soybean lines at 21, 28, 42 and 49 DAP. Plants showed very fast growth at 21 to 42 DAP and slowed down at 49 DAP (Table 2).

The percentage of plant height at 100% field capacity increased by 39.22% and 40.86% at 21 to 28 DAP and 28 to 35 DAP, respectively. The percentage of plant height decreased from 42 to 49 DAP. At the same time, the rate of plant height at field capacity of 75% increased (37.15%) from 21 to 28 DAP and decreased from 35 to 49 DAP. Similarly, the percentage of plant height at 50% field capacity increased by 34.28% at 21 to 28 DAP, and then the rate of plant height has decreased from 35 to 49 DAP (Table 3). Under decreasing the soil’s water potential could be related to decline in the cell enlargement and resulted in reduced growth.

Drought stress inhibited the plant growth including plant height and leaf area. The more level, duration, and frequency of drought stress, the more inhibition was occurred. The high level of drought stress caused a more severe decrease in the chlorophyll content and relative water content in the soybean leaves. Drought stress could increase the osmolyte contents,

Table 1. The scales for *Soybean mosaic virus* (SMV) disease

Scale	Symptom	Reaction of plant
1	Leaf healthy	Very tolerant
2	Mosaic symptom	Moderately tolerant
3	Mosaic symptom with small leaf	Mildly tolerant
4	Mosaic symptom with small leaf and curly	Susceptible
5	Mosaic symptom with small leaf, curly, and stunting	Very susceptible

Table 2. The plant height of soybean lines on the level of drought stress at 21 to 49 DAP

Soybean lines and variety	Plant height (cm)				
	21 DAP	28 DAP	35 DAP	42 DAP	49 DAP
GK/PI	25.3	41.1	50.3	64.6	66.9
GK/M8Grb	22.7	35.9	46.5	59.2	61.5
W/M	21.9	32.8	44.6	53.8	55.7
GK/L-T	24.8	40.9	54.8	67.5	69.2
Anjasmoro	26.6	41.4	57.9	66.1	68.4

DAP= days after planting

antioxidant potential, and peroxidation of the membrane lipids, thus affecting plant growth and development (Dong et al., 2019). The number of fertile nodes had a significant positive correlation ($R^2=0.36^*$) with plant height. The plant height at 49 DAP had the potential for producing high seed weight. Therefore, the plant height on soybean plants triggered the growth of the number of branches per plant, the number of fertile nodes per plant, and the number of pods per plant.

The Number of Leaves. The level of drought stress had no significant effect on the number of leaves at 21 DAP. However, drought stress had a significant effect ($P < 0.05$) on the number of leaves at 28 to 49 DAP. The decrease in leaf number under 50% drought stress was greater (8.7%) than in 75% drought stress (1.8%) at 28 DAP. The leaf number was decreased in 50% drought stress (9.3, 10.5, and 12.7%) which was higher than 75% drought stress (2.6, 4.9, and 5.3%) at 35 to 49 DAP (Table 3). Each of the soybean lines showed a significantly different response in the leaves production. Significant different was also found in the interaction between L (soybean lines) x D (drought stress level). In general, the number of leaves had significantly increased since 21 DAP and peaked at 35 DAP, however the number of leaves was slowly decreased from 42 to 49 DAP (Table 3). Reducing the number of leaves was one of the plant defense mechanism against drought stress (Sacita et al., 2018). A high decrease in the number of leaves indicated a high rate of leaf

senescence (aging), thereby reducing photosynthesis (Wijewardana et al., 2019). In addition, in the reproductive phase, the number of leaves of senescent plants increased. Drought affected the acceleration of senescent leaves, which could shorten the filling period of seeds and produce smaller seeds. Correlations between the number of leaves and seed yielded had a weak positive correlation ($r^2 = 0.21$). This indicated that the higher leaves number in drought stress, the higher the seed weight obtained. The Anjasmoro variety produced the highest number of leaves at 21 to 49 DAP. However, the GK/L-T line maintained the senescence rate since 35 DAP (Table 3).

The 100 Seeds Weight. Drought stress reduced seed yield per plant by an average of 10.08 to 9.25 and 8.47 g/plant at 75% and 50% drought stress, respectively. Drought stress affected the weight of 100 seeds. The 100 seeds weight decreased by 6.9% at 50% drought stress compared to 75% drought stress at 3.5% (Table 4). Drought stress during the seed filling phase affected seed growth rate through photosynthesis. The GK/L-T soybean line had the highest seed weight of 100 grains (16.8 g/plant). Drought stress had affected the percentage of seed weight loss in GK/L-T (0.38%) than in the Anjasmoro variety (7.25%).

Response of the Soybean Lines to the Virus Infection. The GK/L-T reaction was not detected in the presence of SMV and also the lowest Absorbance

Table 3. Effect of drought stress level on plant height (cm) and number of leaves of soybean line

Drought stress level (%)	Days after planting (DAP)				
	21	28	35	42	49
The plant height (cm)					
100	26.40 ± 1.8 a	41.15 ± 3.5 a	56.72 ± 5.7 a	63.26 ± 4.8 a	68.14 ± 1.2 a
75	24.12 ± 1.5 ab	37.24 ± 1.8 ab	50.16 ± 3.1 ab	58.72 ± 1.9 b	63.05 ± 3.6 b
50	22.56 ± 0.7 b	34.06 ± 2.1 b	41.80 ± 0.6 b	50.09 ± 2.3 c	56.90 ± 2.5 c
The number of leaves					
100	5.01 ± 1.4 a	7.96 ± 1.2 a	9.79 ± 1.7 a	9.35 ± 0.5 a	9.12 ± 0.7 a
75	4.88 ± 1.1 a	7.42 ± 0.5 ab	9.15 ± 0.4 b	8.68 ± 0.3 b	8.63 ± 0.2 b
50	4.73 ± 0.8 a	6.21 ± 0.6 b	8.60 ± 0.6 c	8.22 ± 0.3 c	7.91 ± 0.4 c

According to the Tukey Test, values sharing the same letters differ non significantly ($P < 0.05$).

Table 4. Seed weight and 100 seeds weight soybean lines on the level of drought stress

Weight (g/plant)	Drought stress level		
	100%	75%	50%
Seed weight	10.08 a	9.15 ab	8.47 b
100 seeds weight	12.08 a	11.56 ab	11.01 b

According to the Tukey test, values sharing the same letters differ non significantly ($P < 0.05$).

ELISA Value (AEV). The AEV had no significant difference ($p > 0.05$) between the GK/LT and Anjasmoro under drought stress levels (50, 75, and 100%). Moreover, The GK/PI also showed no significant difference ($p > 0.05$) under 75% drought stress level. The accumulation of virus indicated the potential of the GK/L-T line and Anjasmoro variety as inducers of systemic resistance. However, the Anjasmoro was detected in the presence of SMV under 50% drought stress level (Table 5). The mechanism of systemic resistance induction by drought stress treatment and SMV infection was not yet known and still needs to be investigated further. Xu et al. (2008) noted that virus-infected plants before and after drought stress would accumulate salicylic acid, a defense mediator, some osmoprotectants, and antioxidants at high concentrations. The mechanisms of inducing drought tolerance could be activated depending on virus adaptation and the host genotypes. This showed how viruses can promote host survival in situations of drought stress. The GK/L-T line had

the ability as virus inhibitors and tolerant under drought stress. This study confirmed the response of soybean to Soybean mosaic virus infection in drought conditions was diverse within the same species.

The Disease Severity and Seed Yield on Soybean Lines under Drought Stress. Variant analysis test didn't show significant difference between the GK/L-T soybean line and Anjasmoro variety of seed yield components. However, the seed yield of GK/L-T was most superior and had the lowest disease severity under drought stress (Table 6). The results suggested that applying this line could be inducers of systemic resistance under drought stress to SMV. This showed how viruses could promote host survival in situations of drought stress. Thus, SMV infection improved plant tolerance to drought stress. Similarly, in a study conducted by Liu et al. (2004) and Li et al. (2018), virus adaptation and the host genotypes could be activated the mechanisms of inducing drought tolerance.

Table 5. Response of the soybean lines to SMV under drought stress at 49 days after planting (DAP)

Soybean lines and variety	Drought stress level					
	100%		75%		50%	
	AEV ^b	Reaction	AEV ^b	Reaction	AEV ^b	Reaction
GK/PI	1.2 ± 0.6 a ^a	+	0.4 ± 0.1 b ^a	-	0.9 ± 0.1 b ^a	+
GK/M8Grb	1.6 ± 1.2 a	+	1.5 ± 0.8 a	+	2.7 ± 0.2 a	+
WM	1.8 ± 0.8 a	+	1.4 ± 0.2 a	+	2.7 ± 0.5 a	+
GK/L-T	0.2 ± 0.4 b	-	0.1 ± 0.2 b	-	0.3 ± 0.1 b	-
Anjasmoro	0.3 ± 0.8 b	-	0.3 ± 0.9 b	-	0.7 ± 0.3 b	+
Negative control	0.4 ± 0.5 b	-	0.5 ± 0.3 b	-	0.2 ± 0.8 b	-

^aMean ± standart deviation. According to the Tukey test, values sharing same letters differ non significantly ($P < 0.05$) AEV^b= Absorbance ELISA Value; A positive reaction is determined if the Absorbance ELISA Value (AEV) of the test sample is twice the AEV of the negative control.

Table 6. The disease severity and seed yield on soybean lines under drought stress

Soybean lines and variety	Drought stress level					
	100%		75%		50%	
	DS ^a	SY (ton/ha) ^b	DS ^a	SY (ton/ha) ^b	DS ^a	SY (ton/ha) ^b
GK/PI	2.1 ± 0.0 b ^c	0.9 ± 0.5 ab	1.7 ± 0.5 b	1.3 ± 0.1 ab	1.9 ± 0.6 b	0.8 ± 0.5 b
GK/M8Grb	3.0 ± 0.8 a	0.8 ± 0.7 b	2.9 ± 0.0 ab	1.0 ± 0.3 b	3.2 ± 0.4 a	0.9 ± 0.0 b
WM	3.4 ± 0.5 a	0.6 ± 0.2 b	3.3 ± 0.1 a	0.7 ± 0.9 c	3.6 ± 0.0 a	0.9 ± 0.1 b
GK/L-T	1.6 ± 0.4 ab	1.6 ± 0.6 a	1.2 ± 0.0 b	1.8 ± 0.5 a	1.5 ± 0.1 b	1.7 ± 0.2 a
Anjasmoro	1.6 ± 0.2 ab	0.9 ± 0.2 ab	1.3 ± 0.2 b	1.5 ± 0.1 a	1.7 ± 0.4 b	1.2 ± 0.0 a
Negative control	3.5 ± 0.7 a	0.8 ± 0.1 ab	3.5 ± 0.4 a	0.9 ± 0.3 b	3.3 ± 0.8 a	1.1 ± 0.3 ab

DS^a= disease severity; SY^b= seed yield (ton/ha); ^cMean ± standart deviation. According to the Tukey test, values sharing same letters differ non significantly ($P > 0.05$).

CONCLUSION

This study showed the GK/L-T soybean line had the highest seed weight. Drought stress levels had affected the percentage of seed weight loss in GK/L-T than in the Anjasmoro variety. Drought stress inhibited the plant height and the leaf area. The number of leaves slowly decreased from 42 to 49 DAP. Drought stress had affected the percentage of seed weight loss in GK/L-T than in the Anjasmoro variety. However, the level, duration, and frequency of drought stress affected more significantly in the inhibition of the seed filling phase. The GK/L-T reaction was not detected in the presence of SMV and also the lowest of AEV. The seed yield of GK/L-T was most superior and the lowest disease severity under drought stress. The GK/L-T line had the ability as virus inhibitors and tolerant under drought stress.

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AUTHORS' CONTRIBUTIONS

WRA and PGA considered and planned the experiment. WRA, PGA, and ML carried out the Soybean mosaic virus inoculation and screening of the soybean varieties tolerant to SMV including the presence of SMV detection. ML collecting data on agronomic performance of the soybean lines to SMV under drought stress and seed yield at generative period. WRA and PGA collecting data serological and disease severity. WRA, PGA, and ML performing analysis and interpreting the plant damage and resistance under drought stress. WRA prepared the manuscript. The authors provided response and comments on the research flow, data analysis and interpretation as well as shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

All authors declare that we have no conflicts of interest.

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