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Cowpea Mild Mottle Virus (CpMMV) infection and its effect to performance of South Korean soybean varieties

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Abstract. *Sutrisno, Kuswantoro H. 2016. Cowpea Mild Mottle Virus (CpMMV) infection and its effect to performance of South Korean soybean varieties. Biodiversitas 17: 129-133.* Cowpea Mild Mottle Virus (CpMMV) is a very detrimental disease to soybean. This virus infection can cause yield reduction up to 56%. Introduction varieties can be served as alternative material in breeding program to develop resistant variety. This study aimed to evaluate the response of introduction soybean varieties from South Korea to the CpMMV infection. Ten South Korean soybean varieties and two check Indonesian soybean varieties were grown at ILETRI from August to November 2012. CpMMV infection was carried out by natural infection using whitefly (*Bemisia tabaci*) transmission. The results showed that the South Korean variety of Daemang-2 had lower leave malformation level than two Indonesian varieties. Varieties of Cheongja-3, Daemang, Daehwang, Daewon, Danweon, Geomjeong-3 Geonjeongsaeol produced grain yield that equal to Anjasmoro variety. Daepung was the most resistant to CpMMV than other varieties, but having low grain yield. Daemang-2 was the most tolerant to CpMMV, because it still able to produce high grain yield although showing high leaf infection. These two varieties could be used as gene sources for resistant/tolerant to CpMMV in soybean breeding program.

Keywords: Agronomic performance, *Bemisia tabaci*, CpMMV, Korean soybean, yield potential

INTRODUCTION

As long as last decade, demand of soybean in Indonesia increases but national production decreases every year. For instance, in 2005, 2010, and 2015, soybean production 808.353, 907.031, and 982.967 ton whereas consumption level achieved 1.89, 2.65, and 2.6 million ton. As a consequence, Indonesian government had to import as much as 1.08, 1.74, and 1.6 million ton to fulfill this demand (BPS 2015; Faostat 2015). There are many aspects that cause low soybean production in Indonesia. One of the aspects is the limiting soybean production by pest and disease. The high level of whitefly (*Bemisia tabaci*) population is a major constraint in soybean cultivation (Marwoto et al. 2011). This pest is a viral vector of Cowpea Mild Mottle Virus (CpMMV) that attacks the leaf tissue (Laguna et al. 2006). The infected leaves gradually become malformations which ultimately inhibits the growth and yield. The impact of the damage caused by these pests can reduce yields up to 11-56% (Akin 2003).

Various attempts to obtain whitefly resistant variety have be done by exploration, selection of local varieties, crossing (Barmawi et al. 2012), as well as the introduction of new varieties from other country. Introduction of new varieties is often done to increase the diversity of genetic resources. The present of the wide genetic resources diversity is expected to be able to improve varieties for high yielding or pests and diseases resistances. To broaden the diversity of genetic resources, we can introduce soybean germplasm from areas with rich in soybean landraces and wild relative such as China, Japan and Korea.

Adaptation of a plant in new habitat is very important. Therefore, adaptation test is intended to determine plant characteristics expressed in the new area. The performance of the plants generally changes when grown in the new area (Li et al. 2014). If an introduced variety can grow and maintain its characters well, this introduced variety can be used as a new materials for further breeding program based on the superior traits of the variety such as high yielding, large grain size, and pest and disease resistance.

Adaptation test of South Korea introduced varieties that performed in 2012 showed that those varieties have a shorter plant height and lower grain yield than the Indonesian varieties (Kuswantoro et al. 2014). However, the introduction varieties have larger grain size than the Indonesian varieties. This larger grain size trait is expected to be superior traits that can be inherited through a crossing and allows plant genetic improvement for high yielding through large grain size improvement. In addition, other characters such as a resistance to pest and disease are also needed. Some studies reported that whitefly infestation by transmission virus CpMMV causes many types of physiological obstacles such as photosynthesis and enzyme activity inhibition (Zhang and Wen 2008) decreasing chlorophyll leaf index, plant height, number of branches (Jindal et al. 2009), number of pods, (Barmawi et al. 2012), grain size, grain yield (Akin 2003), and acceleration of defoliation (senescence) (Jindal et al. 2009). These physiological obstructions will greatly affect the quality of the grain yield of the genotype. However each genotype has a different response or resistance level to the whitefly

infestation or CpMMV infection. It leads the pest and or disease resistance level of each variety should be tested. This study aimed to evaluate the response of introduction soybean varieties from South Korea to the CpMMV infection.

MATERIALS AND METHODS

The experiment was conducted in Indonesian Legume and Tuber Crops Research Institute (ILETRI), Malang, East Java, Indonesia, since August until November 2012. The tested varieties consist of ten soybean varieties that were introduced from South Korea, i.e. Cheongdu-1, Cheongja-3, Daehwang, Daemang, Daemang-2, Daepung, Daewon, Danweon, Geomjeong-3, Geonjeongsaeol, and two Indonesian soybean varieties i.e. Detam-1 and Anjasmoro). The design was arranged in Randomized Completely Block Design (RCBD) with three replications. Each experimental unit consisted of three polybag with two plants per polybag. Plants were fertilized using NPK fertilizer (16-16-16) at a dose of 2 g/polybag that was applied at seven days after planting (DAP). Watering was given every three days with one liter of water for each polybag. Natural whitefly infestation since 21 DAP until maturing age was used for CpMMV resistance level test. Population of whitefly was controlled with pesticide in order to keep the population between 50 - 100 individual per clump. Plant were harvested when the leaves were fallen, pods color start to be brown, and the pods were dry. Growth component were observed on leaf diseased score, flowering age, harvested age, plant height, number of branches, number of reproductive nodes, pod length, pod width, number of filled pods, number of empty pods, number of grains per plant, percentage of abnormal grains, 100 grains weight and grain weight per plant. The level of infected leaf score was calculated by the following method (Zubaidah et al. 2010):

$$I = \frac{\sum(n.v)}{N.Z} \; x \; 100$$

Where:
I = the infestation intensity per plant (%)
n = number of infected leaves on certain score
v = score category at certain leaf
N = number of observed leaves per plant
Z = the highest score category

RESULTS AND DISCUSSION

The results showed that preference levels of whitefly against all tested varieties that indicated by a leaves score were quite diverse. Performance of agronomic characters and infected level by CpMMV were different among all tested varieties. The level of susceptibility of varieties maybe caused by genetic different.

Flowering ages of introduction varieties were shorter than Indonesian varieties ranging from 4 to 6 DAP. Four introduction varieties, i.e. Cheongja-3, Daehwang, Daemang-2, and Geonjeongsaeol, had equivalent flowering age to variety of Detam-1, while other introduction varieties had longer flowering age. Anjasmoro had the longest flowering age 33 DAP (Table 1). Flowering age of introduction varieties of this study was generally shorter than flowering age in the previous study that was not infested by whitefly. Flowering age in this study was shorter one to two days than previous study except Geonjeongsaeol that had four days longer (Kuswantoro et al. 2014), This fact indicates that whitefly infestation could affect flowering time. Infected of CpMMV through whitefly caused metabolism disturbance, and finally encourage plant to faster flowering. This stress basically effect availability and activity of gibberellin hormone (Hamayun et al. 2010) or gibberellin, abscisic acid, and brassinosteroids (Domagalska et al. 2010).

Table 1. Agronomical characters of South Korean introduced soybean varieties that infected by CpMMV, Malang, East Java, Indonesia 2012

Varieties	Flowering age (DAP)	Harvesting age (DAP)	Plant height (cm)	Number of branch	Number of reproductive nodes	Number of filled pods*)	Number of unfilled pods**)
Anjasmoro	33.2 ^{a}	91.8 ^{a}	57.3 ^{a}	4.4 ^{ab}	20.3 ^{ab}	6.4 ^{ab}	1.8 ^{a}
Detam-1	31.9 ^{ab}	83.2 $^{a-c}$	55.8 ^{a}	6.2 ^{a}	26.9 ^{a}	6.9 ^{a}	1.0 ^{b}
Cheongdu-1	27.0 ^{c}	74.3 ^{cd}	23.3 ^{bc}	3.0 ^{b}	8.3 ^{c}	3.6 ^{de}	1.2 ^{b}
Cheongja-3	28.1 ^{bc}	77.0 $^{b-d}$	30.8 ^{b}	4.8 ^{ab}	9.0 ^{c}	4.0 ^{de}	1.3 ^{ab}
Daehwang	29.4 $^{a-c}$	73.6 ^{cd}	26.5 ^{bc}	4.5 ^{ab}	9.8 ^{c}	3.5 ^{e}	1.2 ^{b}
Daemang	27.2 ^{c}	77.5 $^{b-d}$	18.6 ^{c}	4.8 ^{ab}	9.7 ^{c}	4.1 ^{de}	1.2 ^{b}
Daemang-2	28.5 ^{bc}	85.3 ^{ab}	24.4 ^{bc}	3.8 ^{b}	12.9 ^{bc}	5.4 ^{bc}	1.3 ^{ab}
Daepung	25.6 ^{c}	70.4 ^{d}	22.0 ^{bc}	2.8 ^{b}	9.9 ^{c}	4.3 $^{c-e}$	1.3 ^{ab}
Daewon	25.9 ^{c}	77.3 $^{b-d}$	26.3 ^{bc}	3.5 ^{b}	9.5 ^{c}	4.1 ^{de}	0.6 ^{b}
Danweon	27.1 ^{c}	72.7 ^{cd}	20.4 ^{bc}	4.1 ^{b}	14.7 ^{bc}	4.8 ^{cd}	1.3 ^{ab}
Geomjeong-3	27.7 ^{c}	72.6 ^{cd}	24.7 ^{bc}	4.4 ^{ab}	11.3 ^{c}	3.8 ^{de}	1.3 ^{ab}
Geonjeongsaeol	29.0 ^{bc}	73.6 ^{cd}	21.1 ^{bc}	4.6 ^{ab}	10.6 ^{c}	4.2 $^{c-e}$	1.1 ^{b}
CV	4.63	4.61	13.6	16.4	19.8	8.92	13.1

Note: *) 1× sqrt transformation; **) 2× sqrt transformation. Values followed by the same letter in the same column do not different according to LSD α 0.05

Table 2. Infected leaves score and agronomical characters of South Korean introduced soybean varieties, Malang, East Java, Indonesia, 2012

Varietas	Infected leaf score (%)	Pod length (cm)	Pod width (cm)	Number of seed per plant	Weight of 100 seeds	Abnormal seed (%)	Grain yield per plant (g)
Anjasmoro	50.8	3.7 [a]	0.9 [a]	77.2 [ab]	10.7 [c]	50.2	8.2 [bc]
Detam-1	47.3	3.9 [a]	0.9 [a]	103.7 [a]	12.2 [c]	4.3	12.2 [a]
Cheongdu-1	30.0	4.5 [a]	1.0 [a]	26.6 [ef]	15.3 [bc]	22.1	3.9 [d]
Cheongja-3	52.2	4.3 [a]	1.2 [a]	22.1 [f]	23.9 [a]	32.0	5.5 [cd]
Daehwang	40.3	4.4 [a]	1.2 [a]	22.4 [f]	25.9 [a]	36.7	6.5 [b-d]
Daemang	44.8	4.3 [a]	1.1 [a]	29.3 [d-f]	19.9 [ab]	16.6	5.3 [cd]
Daemang-2	51.5	4.0 [a]	1.0 [a]	56.4 [bc]	19.8 [ab]	17.5	9.4 [ab]
Daepung	22.2	3.9 [a]	0.9 [a]	43.8 [c-e]	11.3 [c]	6.7	4.1 [d]
Daewon	43.3	3.8 [a]	1.3 [a]	28.2 [d-f]	23.8 [a]	14.7	6.1 [b-d]
Danweon	33.1	4.2 [a]	1.0 [a]	48.8 [b-d]	14.5 [bc]	8.3	7.1 [b-d]
Geomjeong-3	46.5	4.3 [a]	1.2 [a]	24.7 [ef]	23.2 [a]	28.4	6.5 [b-d]
Geonjeongsaeol	43.3	3.9 [a]	1.2 [a]	28.7 [d-f]	23.5 [a]	13.2	6.7 [b-d]
CV	50.8	6.61	14.7	9.58	13.1	20.7	19

Note: Value that followed by the same letter did not different according to LSD α 0.05

Harvesting ages of introduction varieties were shorter than Indonesian varieties except variety of Daemang-2. Harvesting age of introduction varieties ranged from 72 to 85 DAP, while Indonesian varieties ranged between 83-92 DAP. Anjasmoro variety had the longest generative phase compared to other tested varieties, followed by Daemang-2 and Detam-1 with 58, 57, and 49 days respectively (Table 1). Daemang-2 that had long generative phase (since flowering until harvesting time) also had longer and maximum pod filling phase. Longer flowering time caused longer harvesting age. Hakim (2012) stated that the lowering date is closely linked to harvest age. Harvesting age in this study seems to be shorter than previous study with harvesting age of 74-84 DAP (Kuswantoro et al. 2014). For example, varieties of Cheongja-3 and Daemang with the most severe infection had harvesting time 5 days shorter than when they were grown in normal condition without whitefly infection, namely 83 and 82 DAP. The level of whitefly infestation that affects plant growth and development may be influence the speed up of harvesting age. Zhang (2008) concluded that offensive of *Bemisia tabaci* inhibits antioxidant enzymes activities that finally increase senescence or defoliation and accelerates plant death.

Plant height of introduction varieties was lower than Indonesian varieties. In general, the Indonesian varieties had double plant height than introduction varieties. The highest plant height of introduction varieties was achieved by Cheongja-3, while the lowest was achieved by Daemang i.e. 30 and 18 cm respectively (Table 1). Infection of CpMMV caused abnormality in plant growth lead hampering plant growth. Other studies stated that virus which spread by whitefly causes lower plant height (Jindal et al. 2009). Some varieties extremely decreased plant height, while others slightly decreased. For instance, variety of Cheongja-3 decreased 1 cm while Daemang decreased 3 cm. The declining of plant height is not always in line with the level of CpMMV infection, but also by genetic factor. For example on the similar leaves score and number of abnormal grain, the declining plant height of the tested genotypes was not similar.

Branches numbers of several introduction varieties were equivalent to Anjasmoro variety but lower than Detam-1 variety. The number of branches in introduction varieties was quite a lot compared to Indonesian varieties despite having shorter plant (Table 1). A large number of branches likely due to stunted plant growth so that vegetative growth directed to establishment of branches. Infestation of CpMMV detained plant growth but likely did not directly affected number of branches. This evidenced with number of branches in this study were equal to varieties when grown normally (Kuswantoro et al. 2014). However, other study stated that whitefly infection diminished branches number (Jindal et al. 2009). The difference occur, probably due to each genotype has different response to whitefly and CpMMV infestations. In this research, number of branches was closely linked to performance of the plant height (Hakim 2012).

The numbers of reproductive nodes of Indonesian varieties were double than the introduction varieties. Reproductive nodes number of introduction varieties of Danweon, Daemang-2, and Geomjeong-3 were 14.7, 12.9, and 11.3 respectively (Table 1). Number of reproductive nodes seemed very affected by plant height and number of branches. Varieties that had higher plant height and more branches tend to generate more reproductive node number. Judge (2012) stated that plant height and branch positively associated with the number of nodes. Higher infection of CpMMV may reduce the number of nodes that produce pods. For instance, varieties of Cheongja-3 and Daemang which looked susceptible to CpMMV infection produced fewer amount of reproductive nodes than Daemang-2. Compared to previous study, reduction of reproductive node number of Cheongja-3 and Daemang was higher than Daemang-2 where it was more resistant to CpMMV infection and had no decreasing reproductive nodes number (Kuswantoro et al. 2014).

Infection of CpMMV diminished both establishment of filled pods and unfilled pods. The moderate resistance variety to whitefly, Daemang-2, produced more number of pods than other introduction varieties. Performance of pods number seems more diverse than previous study (Kuswantoro 2014). This may be affected by plant response to CpMMV. Barmawi et al. (2012) suggested that CpMMV infection that transmitted by whitefly decrease the number of pods. Number of pods of Indonesian varieties seemed more than introduction varieties. Both of Daemang-2 and Danweon varieties produced the most pods than other introduction varieties.

Pods length and width or pods size of all tested varieties did not different but the number of grains, 100 grains weight and grain yield were different on each variety. The amount of grains of Indonesian varieties was higher than introduction varieties. Danweon and Daemang-2 varieties achieved the highest number of seed among tested introduction varieties. Number of grains in each of varieties affected by plant performance i.e. plant height, number of branches, and number of nodes. For example, Daemang-2 variety that has higher plant height, more number of productive nodes and number of filled pods had more number of seeds than others introduction varieties (Table 2). Similar finding also stated by Sarutayophat (2011) and Judge (2012) that the number of grains was positively correlated with its vegetative performance.

Introduction varieties achieved 100 grains weight or grain size larger than Indonesian varieties. Daehwang produced the largest grain size, followed by Choengja-3, Daewon, Geomjeong-3, Geonjeongsaeol, Daemang, and Daemang-2 varieties respectively. Daepung, Cheongdu-1 and Danweon varieties produced the smallest grain size and their sizes were similar to the Indonesian varieties (Table 2). Generally, level of CpMMV infection reduced weight of 100 seeds with varying in decline level. Infection of CpMMV causes photosynthesis rate and photosynthate distribution into seed is hindered and lead smaller grain size. According to Zhang and Wen (2008), infestation of whitefly that transmitted by CpMMV inhibits rate of photosynthesis and activity of antioxidant enzymes. Jindal et al. (2009) suggested that whitefly transmission decreased leaf chlorophyll index. That conclusion is equal to this finding that weight of 100 grains in Daepung and Daehwang only 10 to 25 g respectively whereas in the previous study that grown without whitefly infection reached 19.10 and 33.99 g. In this fact, whitefly infection caused decreasing yield until 33%.

Infection of CpMMV caused different leaf infection. Two Korean varieties i.e. Cheongja-3 and Daemang-2 had higher infected leaf percentage than Anjasmoro and Detam1 whereas other seven Korean varieties had lower infected leaf than Indonesian varieties. Cheongja-3 and Daemang-2 had the highest infection among all tested Korean varieties. However, Daepung had the lowest damage (Table 2). The level of leaf damage was likely affected by thickness and leaf trichomes. Other studies reported that density, length, and stiffness of trichomes affect whitefly preference to infect plant leaves (Taggar and Gill 2012).

CpMMV infection increased percentage of abnormal grain. Anjasmoro varieties seemed the most susceptible while Detam-1 showed the most resistant to CpMMV infection. Cheongja-3 and Daehwang had the highest percentage of grain damage among all Korean varieties while Daepung and Danweon had the lowest abnormal grain percentage. Difference in this abnormality is due to the different resistance of the varieties through the mechanism of metabolic, genetic, physic, as well as interaction among those factors. The level of susceptibility of introduction varieties to disease is caused by environmental factors that different with their original region. Introduction varieties have to adjust with two environmental stresses i.e. abiotic (environmental) and biotic (pest) stresses simultaneously (Mbeyagala et al. 2014).

Infection of CpMMV virus decreased crop yield with different level. The same result also was stated by Akin (2003) and Barmawi (2012) that CpMMV virus infection decrease soybean grain yields. Some varieties having different susceptibility could have similar yield reduction, while varieties with similar susceptibility showed similar yield reduction. Cheongdu-1 and Daemang-2, for example, were equivalent in decreasing yield although they had different susceptibility, whereas Daewon, Danweon, Geomjeong-3, and Geonjeongsaeol that had same susceptibility had different yield. Daewon was the most decreased varieties than their yield when grown in normal condition without CpMMV stressed (Kuswantoro et al. 2014). Grain yield of Indonesian varieties were higher than introduction varieties. However, Daemang-2, the introduction varieties, produced grain yield higher than Anjasmoro but lower than Detam-1. Some of introduction varieties such as Daewon, Danweon, Geomjeong-3, Geonjeongsaeol, and Daehwang had grain yield equivalent to Anjasmoro.

In conclusion, agronomical performance of South Korean introduction varieties that infected by CpMMV seemed to be lower than Indonesian varieties. Infection of CpMMV hampered plant height growth, decreased number of filled pods and grain yield, increased percentage of infected grain, and accelerating harvesting time. All Korean varieties were more resistant than Anjasmoro variety but more susceptible than Detam-1. Variety of Daepung having the lowest infection indicated that this variety was more resistant than other varieties. In the other hand, Daemang-2 seemed to be more tolerant to CpMMV, because although it had high infected leaf score but it was able to produce high grain yield. Therefore, Daepung and Daemang-2 could be used as gene sources in soybean breeding program for resistant/tolerant to CpMMV.

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