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Research Article

The Effect of Silica (SiO_2) to the Severity of Yellow Leaf Curl Disease on Chili Pepper

Dewa Gede Wiryangga Selangga[1)], Sri Hendrastuti Hidayat[1)]*, Anas Dinurrohman Susila[2)], & Suryo Wiyono[1)]

[1)]*Department of Plant Protection, Faculty of Agriculture, IPB University*
Jln. Kamper, Kampus IPB Dramaga, Bogor, West Java 16680 Indonesia

[2)]*Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University*
Jln. Meranti, Kampus IPB Dramaga, Bogor, West Java 16680 Indonesia

**Corresponding author. E-mail: srihendrastutihidayat@gmail.com*

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ABSTRACT

Yellow leaf curl disease of chili pepper caused by *Pepper yellow leaf curl virus* (PYLCV) has been reported as an important disease in Java and Bali. Disease severity reached 80−100% and it may cause significant yield losses. In order to reduce the negative impact of synthetic insecticides, silica application was evaluated for its potency to suppress the disease. A greenhouse experiment was conducted using randomly block design with 2 factors: PYLCV isolate (Java and Bali) and silica (SiO_2) treatment (with and without). Parameters observed were disease symptoms, incubation period, disease incidence and severity, and total silica level. The symptoms of virus infection in Pelita 8 and Seret cultivars were yellow mosaic, leaf curl, green mosaic, dwarf, and cupping upward or downward. A Specific DNA fragment of 912 bp was successfully amplified from 4 samples. Four sequences were obtained and further analysis showed their highest homology, i.e. 96% and 97% with *Pepper yellow leaf curl Indonesia virus*-Java (PYLCIV-Java) (JX416180) and PYLCIV-KrthAl (LC381274), respectively. Infection by different virus isolates did not affect disease severity significantly. The application of silica was able to delay symptom development and to suppress the severity of the disease in the range of 16.67−30.33%. Silica application on the soil increased the total content of silica in the plants. However, a further experiment is required to understand the mode of action of silica in inducing plant resistance to the pathogen.

Keywords: disease severity, incubation period, insect vector, *Pepper yellow leaf curl virus*, sequence homology

INTRODUCTION

Yellow leaf curl is an important disease on chili pepper in Indonesia. Setiawati *et al*. (2008) reported the loss caused by yellow leaf curl disease in Central Java reached 20-100%. This disease spread rapidly in several regions, especially in Central Java, West Java, Yogyakarta, and Lampung. In 2012, the symptoms of yellow leaf curl disease on chili pepper were first discovered in Bali, and further reported by Putra (2015) that the incidence of yellow leaf curl disease on chili pepper in Kertha, Payangan, Gianyar, Bali reached up to 22.75%. In July 2017, a survey conducted in 12 locations in 4 districts of Bali showed that the incidence of pepper yellow leaf curl disease reached up to 100%, while the severity of the disease was 18−87% (Selangga, unpublished data). The cause of yellow leaf curl disease on chili pepper in Java have been identified as *Pepper yellow leaf curl Indonesia virus* (PYLCIV), which is a member of *Begomovirus* and its transmission occurred through vector insects, i.e. whiteflies, *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae) (Sulandari *et al*., 2006). Similarly, the cause of yellow leaf curl disease in Bali was PYLCIV with 95.9% of homology level against PYLCIV isolates of Java (Selangga, unpublished data).

Various efforts have been conducted to control pepper yellow leaf curl disease through modification of cultural practices, such as protecting seedlings using net covers (Yasa *et al*., 2012) and planting barrier crops (Friarini *et al*. 2016). Synthetic insecticides were used to control insect vectors, although they did not give satisfactory results, especially when the insect population is very high (Song & Swinton, 2009). In addition, *B. tabaci* could easily be resistant to synthetic insecticides (Norris *et al*., 2003). Efforts to develop resistant varieties have been carried out, yet until now it has not been successful. Faizah *et al*. (2012) have identified the genotype IPB C12 as a resistance candidate of chili pepper to PYLCV and has the potential to be used as a parent in breeding scheme. The use of plant growth promoting

rhizobacteria and endophytic fungi is neither ineffective to control pepper yellow leaf curl disease (Priwiratama *et al*., 2012; Lestari *et al*., 2018).

This study was conducted to evaluate the potential of silica application in suppressing the severity of yellow leaf curl disease. Previous research reported that thrips (*Frankliniella schultzei*) as an insect vector of *Tomato spotted wilt virus* (TSWV) in Brazil, was able to be suppressed by the application of silica (Almeida *et al*., 2009). The application of silica could also suppress *Pythium aphanidermatum* infected the roots of tomato plants (Heine *et al*., 2007) and *Pyricularia oryzae* caused blast disease in rice (Cacique *et al*., 2013).

MATERIALS AND METHODS

Propagation of Virus Inoculum

The virus isolates used for this study was originated from Brebes, Central Java (isolate G1) and from Payangan, Gianyar Bali (isolate G2). Isolate G1 was a collection of Plant Virology Laboratory, IPB University, while G2 isolates were obtained from the field study in Bali. The two isolates have been identified based on their sequence analysis and their sequence identity have been deposited in GenBank, i.e. PYLCIV-Java (JX416180) and PYLCIV-KrthA1 (LC381274), respectively. Propagation of PYLCIV inoculum was carried out by insect transmission on chilli pepper var Bara.

The whitefly used for insect transmission were from the collection of the Laboratory of Plant Virology, Department of Plant Protection, Faculty of Agriculture, IPB University. *B. tabaci* were reared on cotton plants isolated with gauze cages in the greenhouses. *B. tabaci* was given an acquisition feeding period of 24 hours on chili pepper plants as the initial inoculum source (originated from the field), then transferred to healthy chili pepper plants aged 6 weeks after planting (10 whiteflies/plant) for an inoculation feeding period of 24 hours. After that, *B. tabaci* was removed from the chili pepper plants and the plants were maintained until they exhibited disease symptoms. The symptomatic plants were then used as the virus inoculum in the experiment.

Assay of Silica Application in PYLCIV Infected Plants

Preparation of test plants. Two chili pepper genotypes, i.e. cv Pelita 8 and cv Seret were used for this assay. Chili seeds were sown using commercial media (a mixture of manure, compost, and husk). About 3−5 weeks after sowing or when the seedlings have 3−4 leaves, the seedlings were transferred into a plastic bag (30 cm × 35 cm) which has been filled with a mixture of soil and manure (2:1 b/b) of 5 kg.

Application of silica in test plants. The silica (SiO_2) product used was gray colored granules (RabanaSil) produced by PT Rabana Agro Resources. Silica was dissolved in water at a dose of 0.428 g/l or equivalent with 200 ppm. Then 240 ml of silica solution was poured into each pot at 14 days after planting.

PYLCIV inoculation on chili pepper plants. Chili pepper plants were inoculated 7 days after application of silica. The inoculation of PYLCIV by *B. tabaci* was carried out as described earlier involving 24 hours acquisition feeding period, 24-hour inoculation feeding period, with 10 whiteflies/plant. All insects were removed from the plants after the inoculation period. Test chili pepper plants were observed until they showed typical symptoms of yellow leaf curl disease. Molecular detection was conducted to confirm the presence of PYLCIV infection in symptomatic plants.

Experimental design and observation variables. The experiment was designed using factorial randomized block design with 2 factors. The first factor was virus isolates: PYCLIV Java isolates (G1) and PYLCIV Bali isolates (G2). The second factor was the application of silica: with silica (S) and without silica (TS). Each treatment was repeated 3 times, with 10 plants per treatment. The total plant used was 240 plants. Observations were carried out every week for 1 month, and parameters observed were symptoms type, incubation period, incidence and severity of the disease following protocol as those in the field observations (Adilah & Hidayat, 2014).

Data analysis. Data were analyzed using the F test by SAS (Statistical Analysis System) 9.4.

Molecular Detection of PYLCIV

The order of detection method started with total DNA extraction, followed by DNA amplification, DNA sequencing, and nucleotide sequence analysis. Total DNA extraction from infected plants was carried out using the modified CTAB method (Doyle & Doyle,

1999). Amplification of target viral DNA was carried out using ready to go PCR bead (Amersham Pharmacia Biotech. Inc.) (Rojas *et al*., 1993), with universal primers for Begomovirus, i.e. SPG1 (5'-CCCCK GTGCGWRAATCCAT-3') and SPG2 (5'-ATCC VAAYWTYCAGGGAGCT-3') (Li *et al*., 2004). The DNA amplicons were sent to First Base, Malaysia for sequencing. Sequencing data were compared with sequential database from GenBank (NCBI, 2013) and analyzed using BioEdit V.7.0.5 software program, CLC Sequence Viewer 7, and MEGA 6.06.

Determination of Total Silica Level

Determination of total silica content in the stems and leaves of the test chili plants was carried out using the gravimetric method by Sapei *et al*. (2015).

RESULTS AND DISCUSSIONS

Disease Symptoms

Disease symptoms observed on chili pepper plants of cv Pelita 8 and cv Seret were yellow mosaic, leaf curl, green mosaic, dwarf, and cupping upward or downward. The symptoms caused by PYLCIV Bali and Java isolates was similar. PYLCIV infections on cv Pelita 8 tends to be more severe than those in cv Seret. Leaf curling, yellowing, and dwarfing were found in cv Pelita 8, while the most severe symptoms in cv Seret were yellowing and leaf curling (Figure 1).

Confirmation of PYLCIV Infection

DNA fragments of 912 bp was successfully amplified from 4 symptomatic leaf samples using the universal primer *Begomovirus* SPG1/SPG2 (Figure 2). Sequencing analysis of the sample DNAs

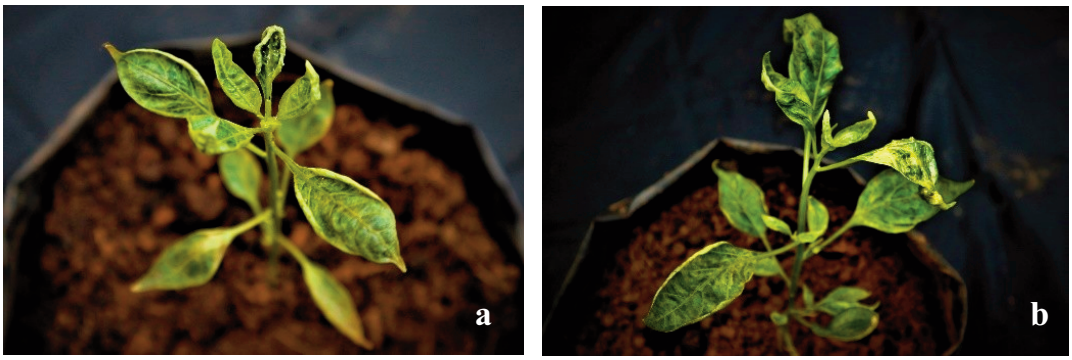

Figure 1. Symptoms of yellow leaf curl disease on (a) cv Pelita 8, i.e. leaf curl, yellow mosaic, and dwarf, and (b) cv Seret, i.e. yellow mosaic and leaf curl

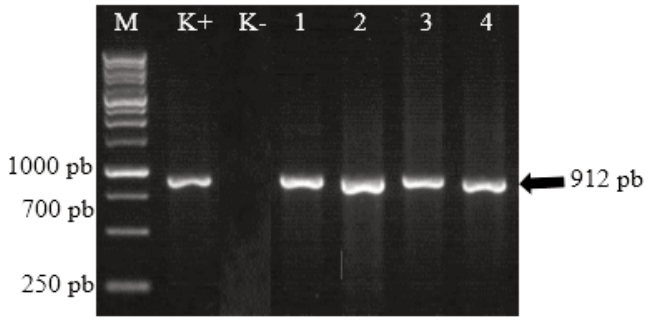

Figure 2. PYLCIV DNA visualization from amplification using SPG1 / SPG2 universal primer on 1% agarose gel [M: DNA marker (1kb ladder); K+: positive control of PYLCIV Tlbg 1 (LC381258); K-: negative control (ddH_2O); sample no. 1 and 2 were plants inoculated by PYLCIV isolates of Java; sample no. 3 and 4 were plants inoculated by PYLCIV isolates from Bali]

confirmed that the virus causing infection on test plants in the greenhouse was PYLCIV with 96% and 97% homology for PYLCIV-Java (JX416180) and PYLCIV-KrthA1 (LC381274), respectively (Table 1).

Absorption of Silica by Test Plants

Preliminary tests were conducted to determine the ability of chili pepper plants to absorb silica. Measurement of total silica levels was carried out per week for one month consecutively. The plants already contained the total silica level of 0.35% and 0.39%, respectively, in cv Pelita 8 and cv Seret, even though the silica was not applied. The total silica level has increased in the first-week observation, 2.39% and 1.92% in cv Pelita 8 and cv Seret, respectively. The application of silica increased the total silica level until the plant reached the initial generative stage at 4 weeks after planting. The total silica level in cv Pelita 8 (7.50%) was higher than those in cv Seret (5.13%) (Table 2). This result confirmed that plants were able to absorb silica applied through the soil starting 1 week after treatment. Thus, further testing to determine the effect of silica on yellow leaf curl disease could be proceeded.

The Effect of Virus Isolate and Silica Treatment on Pepper Yellow Leaf Curl Disease

The use of silica to control plant pests and diseases has not been commonly applied, although silica was known able to stimulate the plant resistance (Gomes *et al*., 2005). The application of silica was able to suppress the development of *P. oryzae* causing blast disease (Wattanapayapkul *et al*., 2011). Almeida *et al*. (2008) showed that the application of silica in Chinese long bean (*Phaseolus vulgaris* L.) was able to increase mortality and reduce oviposition of *B. tabaci*.

Table 1. Homology (%) nucleotide sequences of Begomovirus isolates in the greenhouses with PYLCIV isolates from Java and Bali

Begomovirus Isolates	G1	G2	PYLCIV-Java	PYLCIV-KrthA1
G1	ID			
G2	94	ID		
PYLCIV-Java	96	95	ID	
PYLCIV-KrthA1	92	97	94	ID

Virus incubation period. The first symptom appeared at 7-28 days after inoculation (dai). The longest incubation period was on cv Pelita 8 with treatment of PYLCIV Bali isolate (G2) and silica (S) (14-28 dai), and on cv Seret with the treatment of PYLCIV Javanese isolate (G1) and silica (S) (Table 3). Silica treatment delayed the symptoms or viral infection.

The incidence and severity of the disease. Statistical analysis showed that treatment with silica (S) was significantly affected the incidence and severity of yellow leaf curl disease in cv Pelita 8 and cv Seret. On the other hand, virus isolates and interactions between the two factors did not affect the incidence and severity of yellow leaf curl disease (Table 4 and Table 5). The incidence and severity of the disease in cv Pelita 8 and cv Seret were 83.33−100% and 38.33−68.66%; and 70.0-98.33% and

Table 2. Total silica level in cv Pelita 8 and cv Seret in the preliminary test

Observation (week)	**Level of total SiO_2 (%)***	
	cv Pelita 8	**cv Seret**
0	0.35	0.39
1	2.74	2.31
2	3.99	2.36
3	5.55	3.84
4	7.50	5.13

Remarks: * Total silica was measured based on gravimetric dry weight.

Table 3. PYLCIV incubation period on cv Pelita 8 and cv Seret with silica treatment

Treatment	Incubation period (dai)*	
	cv Pelita 8	cv Seret
PYLCIV Java isolate (G1)		
S (with silica)	7−28	14−28
TS (without silica)	7−21	7−21
PYLCIV Bali isolate (G2)		
S (with silica)	14−28	7−28
TS (without silica)	7−21	7−21

Remarks: * dai = day after inoculation.

42.0−55.00%, respectively. The lowest incidence and severity of the disease was indicated by treatment with silica, hence the silica treatment was considered able to reduce the incidence and severity of the disease in both varieties.

The mechanism of silica to activate plant resistance against pests and pathogens was unknown. According to Correa *et al*. (2005) and Inbar *et al*. (2001), the application of silica was able to activate chitinase, peroxidase, 1.3-glucanase, poly-phenoloxidase, phenylalanine ammonia-lyase enzyme, proteinase, and lipoxygenases in cotton, tomato, and wheat plants. These enzymes have a role in the metabolism of phenolic compounds, such as lignin, which stimulate resistance of plant against pathogens. Makarim *et al*. (2007) reported that silica has a function to strengthen the walls of epidermal tissue and vascular tissue, thus inhibiting pathogenic infections, especially fungi. The thick and strong layer of epidermal tissue may also increase the resistance of plants to pests or inhibit the feeding behavior of pests. Therefore, the potency of silica to control diseases caused by a virus, especially those transmitted by insect vectors, such as yellow leaf curl disease, was needed to be further studied.

Total Silica Levels in Test Plants After Treatment

Silica level in the test plants were measured separately for the stems and leaves, at 4 weeks after planting. Silica level at the stems was higher than those in the leaves, both in cv Pelita 8 and cv Seret. The silica applications in cv Pelita 8 and cv Seret caused an increase of silica level in the stems and leaves (Table 6 and Table 7). The roots have a higher level of silica than those in the stems and leaves indicated that more silica accumulated in the roots and was not rapidly translocated to other parts. Additionally, the chili pepper plants were not known as silica accumulators (Krishardianto & Sukma, 2017), hence the total silica level in the roots did not increase much, even though it was treated with the addition of silica.

Table 4. The effect of virus isolates and silica on incidence and disease severity on cv Pelita 8

Treatment	Disease Incidence (%)	Disease Severity (%)
Virus Isolate		
PYLCIV Java isolate (G1)	90.00	54.66
PYLCIV Bali isolate (G2)	93.33	52.33
F test	NS	NS
Silica Treatment		
S (with silica)	83.33	38.33
TS (without silica)	100.00	68.66
F test	**	**
Interaction of virus isolates and silica treatment	NS	NS

Remarks: NS = not significantly different; **significantly different (P<0.01).

Table 5. The Effect of virus isolates and silica on incidence and disease severity on cv Seret

Treatment	Disease Incidence (%)	Disease Severity (%)
Virus Isolates		
PYLCIV Java isolate (G1)	83.33	46.33
PYLCIV Bali isolate (G2)	85.00	46.33
F test	NS	NS
Silica Treatment		
S (with silica)	70.00	42.00
TS (without silica)	98.33	55.00
F test	**	**
Interaction of virus isolates and silica treatment	NS	NS

Remarks: NS = not significantly different; **significantly different (P<0.01).

Table 6. Total silica level in the stems and leaves of cv Pelita 8 in various treatments

Treatment	Level of total SiO_2 (%)*	
	Stem	Leaf
PYLCIV Java isolate (G1)		
S (with silica)]	1.04	0.53
TS (without silica)	0.35	0.40
PYLCIV Bali isolate (G2)		
S (with silica)	0.39	0.66
TS (without silica)	0.06	0.12

Remarks: *Total silica content was measured based on gravimetric dry weight.

Table 7. Total silica level in the stems and leaves of cv Seret in various treatments

Treatment	Level of total SiO_2 (%)*	
	Stem	Leaf
PYLCIV Java isolate (G1)		
S (with silica)	1.17	0.47
TS (without silica)	0.11	0.15
PYLCIV Bali isolate (G2)		
S (with silica)	1.22	0.62
TS (without silica)	0.13	0.39

Remarks: *Total silica content was measured based on gravimetric dry weight.

The effectiveness of silica to suppress pathogenic infections was determined by the ability of plants to absorb and distribute silica to all parts of the plant (Roesmarkam & Yuwono, 2002). The ability of plants to absorb silica can be differentiated as followed: low (0.5%), i.e. dicotyledons and Leguminosae; moderate (1−3%), i.e. Gramineae such as sugar cane and grasses; and high (10−15%), i.e. wet Gramineae such as rice. Furthermore, Ma *et al*. (2002) stated that although chili pepper was not a silica accumulator plant, its ability to absorb silica was better than other dicotyledons. The use of silica as a strategy to control the pests and diseases in various types of plants was necessary to be optimized through more specific studies.

CONCLUSIONS

The presence of silica in plants was able to increase the resistance of plants to disease, although the mechanism was undetermined. The treatment of granules silica (SiO_2) was able to delay the disease symptoms and significantly influence the incidence and severity of yellow leaf curl disease in chili caused by PYLCIV.

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