Detection Disease of *Sugarcane Streak Mosaic Virus* (*SCSMV*) Via Serological Test on Sugarcane (*Saccharum officinarum* L.), Weed and Insect Vector

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Abstract: ***In Indonesian the research about Sugarcane Streak Mosaic Virus (SCSMV) still not widely practiced, especially in the field of serology as a virus detection. This research was conducted to detect the presence of SCSMV at sugarcane leaf samples healthy, and leaf samples symptomatic SCSMV, pest insect of sugarcane is suspected as a vector, and weeds as indicator plants using antisera rSCSMV-CP from India. The research was conducted on sugarcane cultivation at dry seasons in each experiments field and laboratory Indonesian Sugar Research Institute. Data obtained from antigens reaction were observed qualitatively by looking at the color of change from microtiter plate wells. The results of the analysis are expressed in the form of negative or positive infected SCSMV disease. Measurement of disease severity caused SCSMV calculated using formula KP (Disease Severity) and was calculated the number insect populations found on field. The result of ELISA using antisera rSCSMV-CP at leaf sample infected SCSMV tested positive with the color indicator on the microplate well is yellow, and at leaf healthy sample, insect tested and weeds found around sugarcane otherwise negatively with indicators of well microplate whiter and brighter color. The population density of C. lanigera Zehntner and S. sacchari Cockerell at 10 sugarcane varieties were observed at random in each experimental farm population average number ranged from 39,5-116,3 tail/leaf and 5-14 tail/rod with humidity of 55% and temperature reaching 33^{0}C. Detection using ELISA method can be use for routine detection virus and can tested sample with large scale in a short time and cost of testing procedure is relatively cheaper, efficient, but has the drawback that it is based on the detection of viral coat protein antigenic properties while the basic techniques can be used to detect PCR to detect negative samples and was not detected in ELISA.***

Keywords: *SCSMV* Diseases, *rSCSMV-CP*, ELISA Method.

1. Introduction

Sugarcane crop are important commodities in agriculture that have high economic value and the main ingredient in the production of sugar (Sumastuti, 2009)[1]. The production of sugar can decline when sugarcane particular disease or pest on sugarcane plantations. Causes of decline in sugarcane production due to diseases such *Sugarcane Mosaic Virus* (*SCMV*) and *Sugarcane Streak Mosaic Virus* (*SCSMV*). This disease has the potential to decrease the yield of sugarcane (Zahra, 2009)[2].

Putra *et al*., (2009)[3] *SCSMV* is a new type of virus attack that many sugarcane plantation in some sugar producing countries including the Indonesian state. Basic information about *SCSMV* which includes the distribution and intensity of attacks, nature bioecology and biomolecular virus is still very limited. Characterization *SCSMV* different with *SCMV* although still in the family *Potyviridae*. Visvanathan *et al*., (2013a)[4] based on molecular characterization of genomic *SCSMV*, the new genus name *Susmovirus* proposed later defined as the genus *Poacevirus* in the family *Potyviridae*. The intensity of symptoms can vary in each cultivar, growing conditions, temperature, and strain of virus.

Kristini *et al*., (2009)[5] reported in 2005 *Streak mosaic* disease infect some locations sugarcane crop on java. The survey results indicate that the *Streak mosaic* disease affects nearly all varieties of sugarcane. Although in general the average intensity of the disease remains low in java, but the existence of this disease is necessary because the disease can potentially reduce the production of sugarcane.

Nurhayati (2012)[6] plant viruses can't infected into the host itself, the organism can only enter the host culture through wound or with the help of other organism of plants infected and then transmited to healthy plants. The virus carrying organisms are known as vectors. Some of the suspected vector can transmit the *SCSMV* viruses is aphids, mealybug or whitefly (*Bemisia tabaci*) and mite. The virus is transmitted by aphids mostly caused mosaik disease and categorized as nonpersistent viruses.

2. Research Objectives

This research aimed to detect *SCSMV* on sugarcane leaf sample symptomatic *Streak mosaic*, leaf sample healthy, insect pest of sugarcane is suspected as a vector, and weed as an indicator plant use *rSCSMV-CP* antibody.

3. Research Benefit

The result is expected to be useful as detection *SCSMV* use *rSCSMV-CP* antibody in an effort to prevent and reduce the spread of the virus inoculum in the field.

4. Materials and Methods

The research was conducted on sugarcane cultivation at dry seasons in each experiments field and laboratory of Indonesian Sugar Research Institute (P3GI) Pasuruan East Java at September 2013 to Oktober 2013. Material used include sugarcane leaf sample healthy and leaf sample symptomatic *SCSMV*, pest insect of sugarcane as a vector, and weeds as indicator plant, *rSCSMV-CP* antibody obtained from Sugarcane Breeding Institute Coimbatore India, *alkaline phosphatase conjugate goat anti-rabbit* (Sigma, USA), material ELISA buffer {Blocking solution, Carbonate buffer (pH 9.6, 200 ml), Enzym conjugate buffer, 1xPBST (PBS with 0.05% *Tween* 20), Substrate solution (pH 9.8, 9.7% *Diethanolamine*, 0.5 mg ml^{-1} pNPP)}, while the tool used is microplate ELISA, ELISA washer, eppendorf, mortar and pestle, sentrifuse 4^0C, termoline, multichanel micropipet and microtip, micropipet single, analytical balance, refrigerator 4^0C, stationery, and camera.

4.1 Detection *SCSMV* using ELISA Method

Sugarcane leaf sample symptomatic, leaf sample healthy, insect, or weeds which serves as an antigen in experiment, each extracted use mortar and added 1:10 w/v carbonate buffer (15mM *sodium carbonate*, 35mM *sodium bicarbonate*, 0.02% *sodium azide*, *Polyvinyl Pyrolindone* 0.5g, pH 9.6), while stem sap obtained by manually pumping sugarcane at age 6 months. Sentrifuse extract sample at 10,000 rpm for 10 minutes and supernatant was taken (Supernatant can be stored at -80^0C until use). The polystyrene plates to be coated with 100 µl supernatant to respective wells as triplicates. Incubate the plate at 37^0C for 2 hour (alternatively overnight at 4^0C) for the binding of the antigen on the plate walls. Later 100 µl blocking solution (100ml *1xPBST*, 2g *Albumin*) was added and the plate are to be incubated overnight at 4^0C (alternatively at 37^0C for 1 hour). Coat the plates with 100 µl *rSCSMV-CP* antibody (raised in rabbit) at a dilution of 1:1500 in conjugat buffer (100ml *1xPBST*, 0.2g *Albumin*) and later with 100 µl *alkaline phosphate conjugate goat anti-rabbit antibody* (Sigma, USA) at a dilution of 1:10,000 and incubate at 37^0C for two hour (alternatively overnight at 4^0C) during both the steps. Between each coating of antigen, blocking solution, antibody and conjugate, the plates are washed thrice with 1xPBST buffer for 5 minutes each. Washing step is performed in order to remove the unbond materials. It is generally done thrice with *Phosphate buffer saline* with Tween 20 in order to maintain isotonicity, since most of the antigen-antibody reaction are optimal under such conditions. The reaction was developed by adding 100 µl of substrate solution and kept at room temperature for 30 minutes until sufficient color development is observed. If the process is rapid 3M NaOH can be added to arrest it. Examination resistance of sugarcane varieties to streak mosaic do with *Scrubbing* method that is by rubbing a cloth dipped in the coir *SCSMV* inoculum solution. Observed symptoms began at age 2 MAP (Month After Plant) to 4 MAP in sugarcane leaves. Measurement of disease severity caused *SCSMV* calculated using formula *KP* (Disease Severity) and was calculated the number insect populations found on field. The results of the analysis are expressed in the form of negative or positive infected *SCSMV* disease (Visvanathan *et al*., 2013a)[4].

5. Results and Discussion

SCSMV was found in each experiments field and cultivation plant collection P3GI on infected leaf indicated by streak mosaic symptoms and the more severe symptoms be marked with the loss of chlorophyll color so leaf color becomes more pale and yellowish and be striped, when in the sun then overlay the color of the leaves become more transparent, whereas in healthy uninfected leaf still look green (Figure 1).

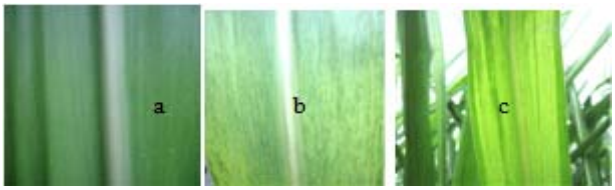


Figure 1: Sugarcane Leaf Healthy and infected leaf (*SCSMV*). a. Leaf healthy; b. *SCSMV* Infected leaf; c. *SCSMV* infected leaf severe

Observations disease severity of *SCSMV* in experiments farm P3GI started at 2 MAP. The percentage of disease severity in the field can vary due to differences resistance of sugarcane varieties were tested against *SCSMV* disease. Sugarcane varieties resistance level of SCSMV disease severity can be categorized: very susceptible (>40%), susceptible (11%-40%), rather susceptible (6%-10%), resistant (1%-5%), very resistant (0%). Based on observations in experimental field varieties are categorized highly susceptible to infected *SCSMV* is KB 08-15, KB 14-16, KB 15-16, KB 18-27, PS 92-1871, whereas very resistant varieties is KB 08-2, KB 13-25, BS 21-22, KB 08-48, KB 15-13, BS 21-17, KB 15-14, KB 24-23 (Table 1). Viral have variety effects on plants due to the nature of the virus has the capability of high infectiousness that infection occurs is rapid and can reach epidemic levels. Bos (1983) in Muis (2002)[7]. Virus infection at a younger age of the plant will cause the plants to be more susceptible than older plant age (Solikhah, 2009)[8].

Table 1: Percentage Average Severity *SCSMV*

No	*Varieties*	*Observation*		
		2 MAP	*3 MAP*	*4 MAP*
1.	KB 08-3	8.4	25.3	25.3
2.	KB 08-15	100	100	100
3.	KB 08-37	1.4	2.8	2.8
4.	KB 08-38	2.7	16.2	16.2
5.	KB 08-2	0	0	0
6.	KB 14-16	44.9	47.8	49.2
7.	KB 14-25	0	4.1	4.1
8.	KB 15-18	2.5	3.8	5.1
9.	KB 15-16	100	100	100
10.	KB 15-17	3.2	13.1	14.7
11.	KB 18-27	100	100	100
12.	BS 04-1	3.2	6.5	6.5
13.	BS 21-4	14.8	27.7	27.7
14.	BS 21-21	12.2	15.7	15.7
15.	BS 21-22	0	9.5	9.5
16.	PS 92-1871	91.8	95	96.7
17.	VMC 76-16	5.8	5.8	5.8
18.	PS 864	18.1	22.7	22.7
19.	KB 08-6	18.1	21.8	21.8
20.	KB 08-48	0	4.8	4.8
21.	KB 15-11	1.6	5	5
22.	KB-15-13	0	0	0
23.	BS 21-17	0	0	1.5
24.	BS 21-32	10.1	15.2	16.9
25.	KB 08-19	10.9	12.5	12.5
26.	KB 08-16	1.5	7.8	7.8
27.	KB 18-28	29.4	39.2	39.2
28.	KB 15-14	0	1.5	4.6
29.	KB 15-10	10.2	26.4	26.4
30.	KB 24-23	0	1.6	3.3

SCSMV = *Sugarcane Streak Mosaik Virus*, MAP = Month After Plant. (Source: Data P3GI, 2013. data not publish).

rSCSMV-CP antisera testing on samples of symptomatic leaf streak mosaic that is varieties PS 864, KB 08-38, and PS 92-1871 shows positif result with color on microplate wells colored yellow, it shows that symptomatic sample tested contained *SCSMV*, increasingly concentrated yellow color on microplate wells then higher *SCSMV* protein concentrate in sample (Figure 2).

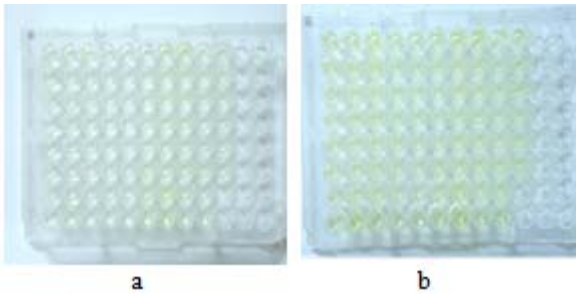


Figure 2: ELISA Test Result After Added Substrat Solution; a**.** Result 1 minutes after added substrat solution; b Result 30 minutes after added substrat solution

Visual observation on microplate wells not give a different significant in color, but can different when read use ELISA reader, the higher value of absorbance that sample tested is positive, but the lower value of absorbance that sample tested is negative (Table 2). According to the research results Visvanathan *et al*., (2013b)[9] ELISA testing is showing positive sample if the absorbance value is more than 0.100, whereas negative sample if absorbance value is less than 0.099 (Table 2).

Table 2: Absorbance Reading Value Use ELISA Reader

No.	*Sample*	*DAC-ELISA reading (absorbance at 405 nm)*	*Info*
1.	Aquades (+D)	-0.093	(-)
2.	KB 15-31 (+D)	-0.143	(-)
3.	PS 864 (daun) (+D)	0.151	(+)
4	PS 864 (nira) (+D)	0.416	(+)
5.	KB 08-38 (daun) (+D)	0.070	(-)
6.	KB 08-38 (nira) (+D)	1.420	(+)
7.	PS 92-1871 (daun) (+D)	0.187	(+)
8.	PS 92-1871 (nira) (+D)	0.304	(+)
9.	*C.Lanigera* (KB 08-38) (+D)	-0.221	(-)
10.	*C.lanigera* (PS 92-1871) (+D)	-0.132	(-)
11.	*S. sacchari* (+D)	-0.073	(-)
12.	*R. exaltata* L. (+D)	-0.323	(-)
13.	*L.javanica* Bth (+D)	-0.386	(-)
14.	*C. aspera* Koen (+D)	-0.010	(-)
15.	KB 15-31	-0.427	(-)
16.	PS 864 (daun)	0.207	(+)
17.	PS 864 (nira)	0.303	(+)
18.	KB 08-38 (daun)	0.116	(+)
19.	KB 08-38 (nira)	1.451	(+)
20.	PS 92-1871 (daun)	0.155	(+)
21.	PS 92-1871 (nira	0.296	(+)
22.	*C.Lanigera* (KB 08-38)	-0.111	(-)
23.	*C.lanigera* (PS 91-1871)	-0.087	(-)
24.	*R. exaltata* L.	0.007	(-)
25.	*L.javanica* Bth	-0.154	(-)
26.	*C. aspera* Koen	0.022	(-)

Negative control = Sample KB 15-31; (+) = Positive *SCSMV*; (-) = Negative *SCSMV*. Value at ELISA Reader are Average of triplicates. (+D) = Plus *Diethanolamine*.

rSCSMV-CP antisera testing on negative samples use sugarcane leaf that is free from *SCSMV* infection and aquades as a negative control showed negative results are markedin the wells whiter and brighter after 30 minutes of added substrat solution. Based on these results that the varieties KB 15-3 does't contain the protein particles *SCSMV* (Figure 2). Insect vectors of suspected as disease *SCSMV* found in P3GI field is *Ceratovacuna lanigera* Zehntner and *Saccharicoccus sacchari* Cockerell (Figure 3). Extract samples of insects used as antigen test material is from ordo Homoptera suspected as vectors of *SCSMV*. Sampel using insects found in sugarcane leaf and stems symptomatic.



Figure 3: Insect of Sugarcane is Suspected as a *SCSMV* vector. a. Imago *Ceratovacuna lanigera* Zehntner and (x) Imago magnification microscopic; b. Imago *Saccharicoccus sacchari* Cockerell and (y) Imago magnification microscopic.

Testing of insect samples using *rSCSMV-CP* antibody on *C. lanigera* and *S. sacchari* showed negative results in

microplate wells (Figure 2.). Based on these results it is suspected that *C. lanigera* and *S. sacchari* sample does't containing *SCSMV* viruses or there is a possibility of a very low content of virus concentration so the value of the readings use ELISA reader is negative and *C. lanigera* dan *S. sacchari* an insect vector which is non persistent. According to the research results Putra *et al*., (2009)[3] stated that population *C. lanigera* and *Rhopalosiphum maydis* can't transmited *SCSMV* on sugarcane. The results of the research abroad also no one has reported presence of insects thatact as *SCSMV* vectors, but the existence of other fleas in sugarcane field is also a concern primarily associated with weed.

Table 3: Average Density Population of *S. sacchari* and *S. sacchari*

No	*Varieties*	*Average Density Population*	
		C. lanigera (tail/leaf)	*S.sacchari (tail/rod)*
1.	KB 08-38	116.3	10
2.	PS 92-1871	47.4	7
3.	PS 864	76.5	14
4.	KB 18-28	95.4	5
5.	KB 14-16	90.6	-
6.	BS 21-4	76.4	8
7.	KB 15-16	95	-
8.	BS 21-32	39.5	-
9.	BS 21-21	42.4	5
10.	KB 15-10	47.5	-

The Population density of *C. lanigera* dan *S. sacchari* on 10 sugarcane varieties random orserved in the experimental field respectively the average number of population ranged from 39,5-116,3 tail/leaf and 5-14 tail/rod with humidity of 55% and temperature reaching 33^0C (Table 3).

The population of *C.lanigera* generally located on leaf of sugarcane, live in groupsin the lower leaf surface and suck the fluid leaf. The lice produce honey dew that causes the leaves covered beneath a black sooty mold which can inhibit the process of photosynthesis. In severe attacks can reduce the yield by 4 points and inhibits the growth process usually occur at the beginning or end of the rainy season. The population of *S. sacchari* found generally located in the middle part of the sugarcane segments to 5 and at the bottom sugarcane. Nymphs of this pest is in the lower stem moves slowly upward. Insects clustered around the ring grows (stem segments) and suck fluid or stem sap (Achadian *et al*., 2011)[10]. Alternative host *C. lanigera* is from famili Poaceae like bamboo and some grasses. There are some records that colonies of this lice found on plants famili *Bixaceae* and *Combretaceae*, but in Japan this spesies reported in *Miscanthus* not in sugarcane Joshi *et al*., (2004)[11].

Some samples of weeds as indicator plants taken at random on the *SCSMV* infected area of sugarcane cultivation is *Rottboellia exaltata* L., *Leucas javanica* Bth., *Cleome aspera* Koen., (Figure 4).

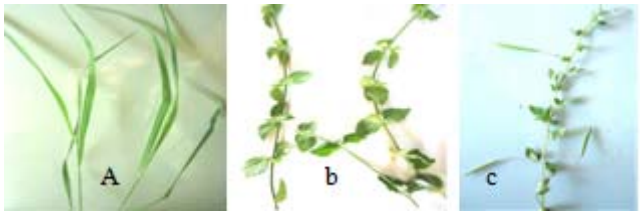


Figure 4: Weed Associated with Sugarcane *SCSMV* Infected at P3GI Experiment Field. a. *R. exaltata* L.; b. *L. javanica* Bth.; c.*C. aspera* Koen.

Testing samples of weeds *R. Exaltata* L., *L. javanica* Bth., *C. aspera* Koen., using *rSCSMV-CP* on microplate wells showed results negative reaction, it is presumably because the samples tested contained no viral proteins or viral concentration in sample contained very limit so that absorbance values at ELISA reader showed negative (Figure 2), otherwise it is an alternative host of *SCSMV* is specific. According to the research results Putra *et al*., (2009)[3] *SCSMV* can only infect sorghum var Rio, sweet corn, and weed *Dactyloctenium aegyptium*. *SCSMV* is a viral particle that does'nt have a broad host range. Symptoms that appear no sorghum and sweet corn like symptoms on sugarcane streak mosaic, whereas on *D. Aegyptium* there are no outward symptoms spesific, but viral particle can detection using RT-PCR method. According to the research results Zahra (2009)[2] *SCSMV* can infection weed *Imperata cylindrica*, *Pennisetum purpureum*, *Paspalum dilatatum* dan *Echinochloa* sp. Statistical data showed no apparent effect on the weeds *SCSMV* infection were tested by streak mosaic symptoms on leaves, but based on whether the symptoms appear on the leaves streak mosaic infection SCSMV differences were tested against weeds.

SCMV detection with (*reverse transcriptase*) RT-PCR technique using specific primers S400-551 with PCR product 359 bp showed that no detection *SCMV* in sample *SCSMV* symptomatic. This means that steak mosaic disease found in field caused *SCSMV* not a mixed infection with *SCMV* (Putra *et al*., 2009)[3]. The results of the research with RT-PCR technique using spesific primer pairs *SCSMV-547F* and *SCSMV-AP3* with PCR product 500 bp also mentions that streak mosaic virus found from survey location positives caused by *SCSMV* (Damayanti *et al*., 2007)[12]. Filogenetic analysis *SCSMV-Idn* (Indonesian) has the highest homology with *SCSMV* from Pakistan in the amount of 98.1%.

Viral detection using ELISA method can be used to detect in large scale on sugarcane virus with relatively cheaper cost and faster, whereas PCR technique can be used to detect negative sample and can't detect with ELISA technique. Results of testing *rSCSMVcp* antisera very efficient for detecting *SCSMV* samples to detect infection naturally infected sugarcane or symptomless in the field. Streak mosaic is viral disease which has the most extensive deployment and a quarantine disease of sugarcane for germplasm exchange and cause significant yield reduction. ELISA detection method is the most common method is preferred because the procedure is relatively simple, reliable, inexpensive and can be tested large scale sample in a short time, but it also has the drawback that it is based on the detection of viral coat protein antigenic properties (Visvanathan *et al*., 2013b)[9].

6. Conclusions and Recomendations

6.1 Conclusions

Based on the research results, we can conclude some of the following:

1. *SCSMV* can detect in sugarcane leaf sample and nira stem rod via ELISA testing use *rSCSMV-CP* antisera from India.
2. *Ceratovacuna lanigera* Zehntner and *Saccharicoccus sacchari* Cockerell insect tested is not *SCSMV* disease vector.
3. *Rottboellia exaltata* L., *Leucas javanica* Bth., *Cleome aspera* Koen., weed tested is not alternate host plant disease *SCSMV*.
4. Detection using ELISA technique can be use for routine virus detection and can test large scale sample in a short time as well as the cost of the testing procedure is relatively cheaper.

6.2 Recomendations

Based on the research conclusions, it can be drawn some recomendations, that are:

Detection virus using ELISA technique still have some weaknesses that the results are still less accurate when compared with PCR method for detect the presence of pathogens. It is necessary for advanced research in serology for improvement ELISA method.

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