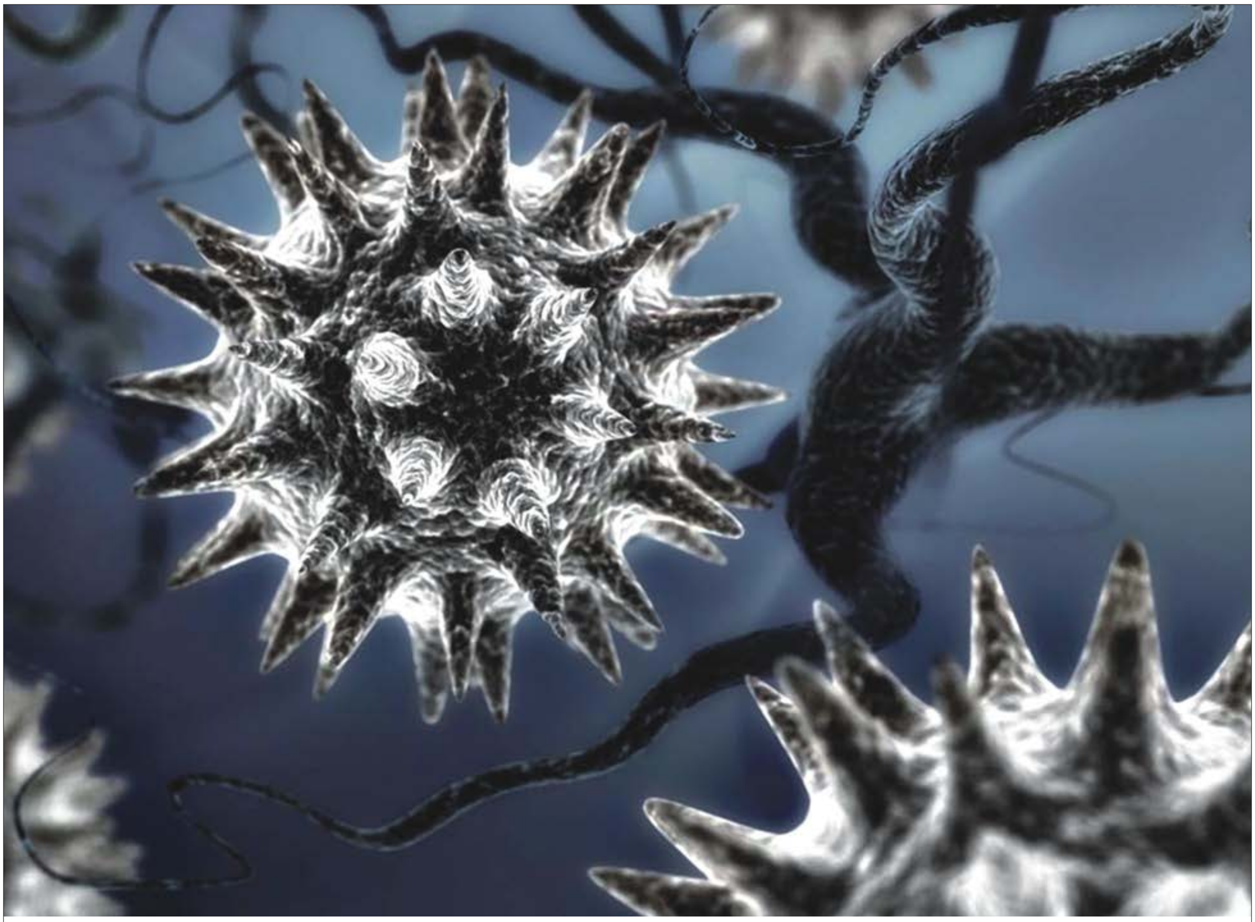

International Journal of

Virology

ISSN 1816-4900

Academic
Journals Inc.

www.academicjournals.com

International Journal of Virology 11 (1): 32-40, 2015
ISSN 1816-4900 / DOI: 10.3923/ijv.2015.32.40

Dispersal, Yield Losses and Varietal Resistance of *Sugarcane streak mosaic virus* (SCSMV) in Indonesia

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ABSTRACT

Sugarcane streak mosaic virus (SCSMV) is a new virus affecting sugarcane plantation in Indonesia. Since the first report in 2005, the virus was widely distributed across Java Island. A field survey was conducted to monitor the expansion of SCSMV distribution in sugarcane plantation and to estimate the impact of SCSMV infection on cane yields. The results of the survey revealed that there was a more widespread occurrence of SCSMV. The virus has infected sugarcane plantation at Jatitujuh SF and Subang SF and this was the first report of SCSMV in that area. SCSMV infection rate also increased with disease incidence ranging from 0.44-86.75%. The rapid distribution of SCSMV was due to the transmissibility of SCSMV through cane cuttings and transportation of planting materials from one area to other areas regardless the health of cane cuttings. The widespread occurrence of SCSMV was also related with the dispersal of variety PS 864 which is highly susceptible to SCSMV. Infection of SCSMV caused a significant reduction to cane yields and also affected cane growth particularly on stalk diameter and number of stalk per stool. To control and prevent the further virus distribution, the use of virus-free cane cuttings and planting resistant variety is recommended. Some promising varieties such as PS 05-103, PS 06-181 and PS 04-526 could be projected to replace the susceptible varieties.

Key words: *Sugarcane streak mosaic virus,* distribution, yield losses, varietal resistance

INTRODUCTION

Sugarcane Streak Mosaic Virus (SCSMV) is a new virus of sugarcane that has been reported in several Asian sugar producing countries including Bangladesh, India, Pakistan, Sri Lanka, Thailand, Vietnam (Chatenet *et al.*, 2005) and Indonesia (Damayanti and Putra, 2011). SCSMV is a member of *Poacevirus*, a new genus in the family *Potyviridae* (Li *et al.*, 2011).

SCSMV was first reported by Hall *et al.* (1998), who identified the virus causing mosaic symptom on quarantined sugarcane germplasm from Pakistan. In India, Hema *et al.* (1999) reported the presence of SCSMV on sugarcane in 1999 and since then the virus was distributed widely across sugarcane-growing states of India. The incidence of SCSMV in the field was more prevalent than mosaic disease caused by *Sugarcane mosaic virus* (SCMV) (Rao *et al.*, 2006; Viswanathan *et al.*, 2007).

In Indonesia, SCSMV was first reported in 2005 when the high incidences of mosaic symptom were found on several sugarcane plantations in East and Central Java. Several commercial cane varieties particularly variety PS 864 which are resistant to SCMV seemed to be severely affected by the disease. Identification using Polymerase Chain Reaction (PCR) technique revealed that most of the mosaic symptoms were positively caused by SCSMV (Kristini *et al*., 2006).

Since the first appearance in 2005, SCSMV is now widely distributed over the commercial sugarcane in Java. A field survey in 2007 showed that SCSMV was observed in 38 cane fields of 5 Sugar Factories (SF) in East and Central Java with disease incidence ranged from 0,28-68% (Damayanti and Putra, 2011). The next survey conducted in sugarcane area of 30 SF in Java during milling season 2008/2009 reported that the disease was observed in 28 SF and only Jatitujuh SF and Subang SF in West Java were free from SCSMV (Putra *et al*., 2014)

SCSMV infected most of commercial sugarcane varieties such as PS 851, PS 862, PS 864 and PSJT 941 and the virus predominantly infected the highly susceptible variety PS 864 (Damayanti and Putra, 2011; Putra *et al*., 2014). It was estimated that SCSMV affected more than 30% of sugarcane areas in Java (Putra *et al*., 2014). The impact of the wide occurrence of SCSMV to sugarcane yield is unknown. This observation was conducted to monitor the recent widespread distribution of SCSMV in Indonesia and the impact of SCSMV infection on cane yields. The result of SCSMV resistance trial of several promising varieties was also reported.

MATERIALS AND METHODS

Distribution survey of SCSMV: The objective of the survey was to monitor the expansion of SCSMV in the fields. The survey was conducted in 2011 at 52 cane fields of 8 locations in Java Island including Pasuruan, Sidoarjo, Madiun, Pati, Pemalang, Yogyakarta, Majelengka and Subang. The 8 locations covered sugarcane plantations of 11 SF namely: Kedawung SF, Candi Baru SF, Watutulis, SF, Purwodadi SF, Sudono SF, Rejoagung SF, Trangkil SF, Sumber harjo SF, Madukismo SF, Jatitujuh SF and Subang SF. During the survey, disease incidences were observed using a method developed by Irawan *et al*. (1999), the attributes of cane fields were recorded and symptomatic leaves were collected.

RT-PCR of SCSMV: RT-PCR test was applied to determine the presence of the virus in the collected leaf samples. A single direct tube method adapted from Suehiro *et al*. (2005) was used for total RNA extraction. RT-PCR using forward primer SCSMV cpF (5'-GTGGGTTCAGTTCTCGGTTC-3') and reverse primer SCSMV-AP3' (5'-TTTTTTCCTCCTCACGGGGCAGGTTGATTG-3') was carried out to amplify a 500 bp DNA fragment of partial coat protein gene (CP) and the 3' terminal of SCSMV. The thermal cycles of PCR were 35 cycles at 94°C for 30 sec, 47°C for 1 min, 72°C for 2 min and a final extension on 72°C for 10 min (Damayanti and Putra, 2011).

Field observation of yield losses: A field observation to estimate the impact of SCSMV infection on cane growth and yield was carried out in cane fields of Watutulis SF. Three varieties i.e. PS 862, PS 864 and PS 881 were observed and five commercial cane fields of each variety were selected as cane field samples. Several parameters were observed at each field sample, including disease incidence at 3 and 6 months after planting; growth parameters i.e., height, diameter and number of stalks at 3, 6, 9 and 12 months after planting and yield parameters i.e., cane weight and rendement at harvesting (±12 months after planting). For observation of the growth parameters, 10 diseased stalks and 10 healthy stalks were selected and for the yield parameters 60 diseased stalks and 60 healthy stalks were sampled. The observed parameters of the diseased and healthy cane samples were compared to estimate the impact of SCSMV infection on cane growth and yield.

Varietal resistance trial: The resistance trial was carried out at Indonesian Sugar Research Institute (ISRI), Pasuruan to obtained new resistant commercial varieties against SCSMV. Eighteen promising cane varieties were tested in this trial including PS 92-750, PS 92-752, PS 04-526, PS 05-103, PS 05-105, PS 05-116, PS 05-119, PS 05-130, PS 05-144, PS 05-175, PS 05-317, PS 05-382, PS 06-155, PS 06-156, PS 06-181, PS 06-196, PS 06-326 and PS 06-346. Variety PS 864 was used as control because this variety is highly susceptible to SCSMV (Putra *et al.*, 2014). A randomized block design using 4 replicates was used in this experiment. Each plot contained of a 3 m row and 10 two-eyes budded cuttings were planted in each row. A standard cultivation was applied during the trials.

SCSMV inoculum was obtained from infected variety PS 864 at the Pathology Collection Farm of ISRI. Young infected leaves were blended in 0.01 M potassium phosphate buffer pH 7.0 (1 g leaf/4 mL of buffer), the sap extract was filtered through cheesecloth and stored at 0-4°C for no longer than 1 h. The sap was then used immediately for the mechanical inoculation and the viral inoculum was placed in an icebox during inoculation. An abrasive pad rubbing method referred to Srisink *et al.* (1994) was used for SCSMV inoculation at 1 month after plating. All stools of each variety were mechanically inoculated using the method.

Disease incidence was observed at 1-3 after inoculation and the appearance of streak mosaic symptom on young leaves was visually examined. Leaf samples of several varieties showing the symptoms were tested using RT-PCR technique to confirm the presence of SCSMV. Resistance level of the tested varieties was classified using the following criteria (Putra *et al.*, 2014): <1% highly resistant, 1-10% resistant, 10.1-20% moderate, 20.1-40% susceptible and >40% highly susceptible.

RESULTS

SCSMV distribution: The results of the survey revealed that SCSMV symptoms were found at the 8 locations in Java and covered 11 Sugar Mills (SF) including Kedawung SF at Pasuruan, Watutulis SF and Candi Baru SF at Sidoarjo, Rejoagung SF, Sudono SF and Purwodadi SF at Madiun, Trangkil SF at Pati, Sumberharjo SF at Pemalang, Madukismo SF at Yogyakarta, Jatitujuh SF at Majalengka and Subang SF at Subang with the average of disease incidence varied (Fig. 1). The symptom of SCSMV infection consisted of a combination of yellow and green short patches on leaf lamina. The symptom was easily recognized on the spindle leaves and the first leaf with the dewlap visible (FVD leaf) and it tend to disappear on the older leaves. Visualisation of mosaic symptoms in the field was varied from mild until severe symptoms depend on the response of variety and the disease severity (Fig. 2). The results of RT-PCR test confirmed that the symptomatic leaf samples collected during the survey was caused by *Sugarcane streak mosaic virus* (SCSMV) (Fig. 3).

About 75% of the 52 observed cane fields were found to be infected by SCSMV with disease incidence ranging from 0.44-86.75%. The highest disease incidence occurred in Kedawung SF in Pasuruan, while the lowest occurred in Subang SF in Subang. At Pasuruan and Sidoarjo all observed fields were affected by the virus with the average of disease incidence 35.1 and 25.94%,

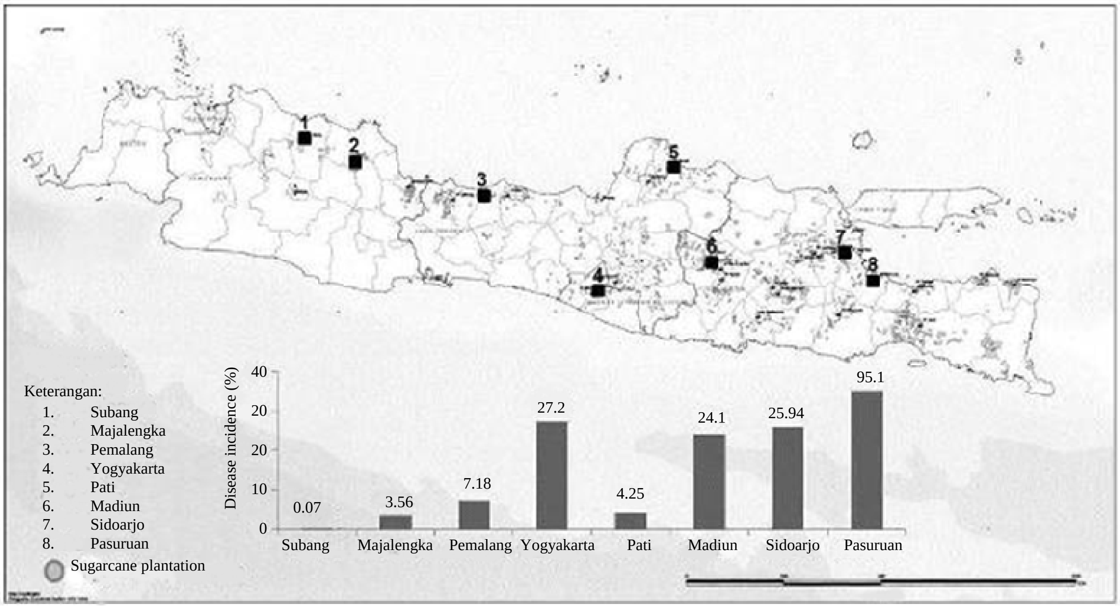

Fig. 1: Map of SCSMV distribution in 8 locations and the average of disease incidences

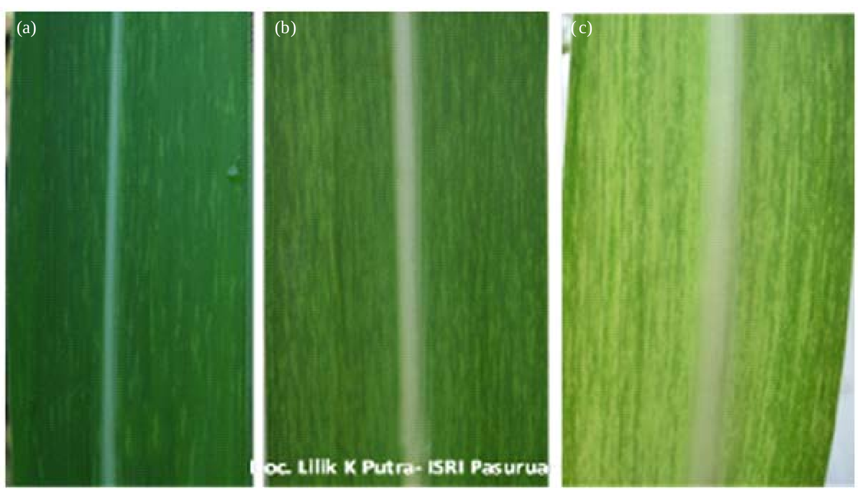

Fig. 2(a-c): Symptom of SCSMV infection on sugarcane leaf, (a) Mild symptom, (b) Clear symptom and (c) Severe symptom

respectively. At Yogyakarta the average of disease incidence was also high reached 27.2%. In general disease incidences in East Java were higher than in Central and West Java.

Comparing to the previous survey, the results of this survey showed that there was an increase of the disease distribution in the field due to the findings of SCSMV in sugarcane plantations of Jatitujuh SF and SF Subang. The survey in milling season 2008/2009 reported that Jatitujuh SF and Subang SF were free from SCSMV (Putra *et al*., 2014). Therefore, at least within 3 years

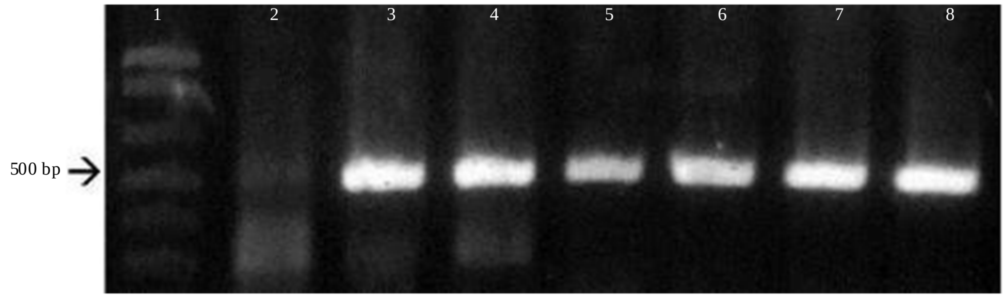

Fig. 3: RT-PCR gel electrophoresis of several leaf samples from the survey, (1) Ladder DNA, (2) Control-, (3) Control +, (4) PS 864, (5) PS 862, (6) BL, (7) KK and (8) PS 881

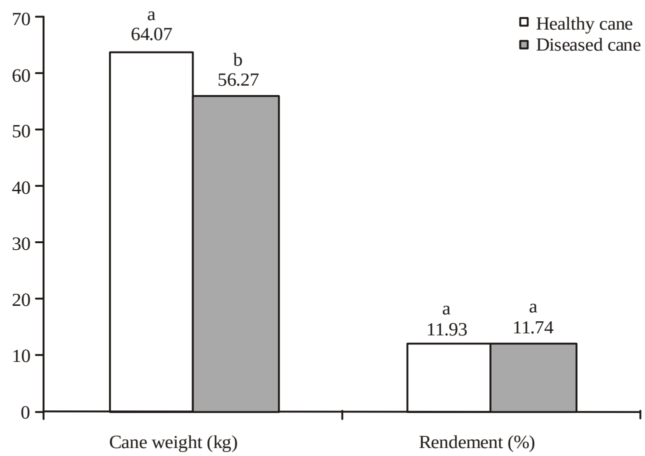

Fig. 4: Cane weight and rendement of healthy and diseased cane. For each parameter, numbers in the same column followed by the same letter was not significantly different (α = 0.05) according to LSD test

SCSMV expansively spread to sugarcane plantation at Jatitujuh SF and Subang SF in West Java. SCSMV infected most of commercial varieties including PS 862, PS 864, BL, PS 881, PSJT 941, PS 951, GMP 2 and KK. The disease was more prevalent in variety PS 864 with disease incidence ranged from 0.46-86.75%. The disease could be found both in plant cane and ratoon cane. There was no difference on the rate of disease incidence between the two plant categories. The disease incidence in irrigated area was mostly higher than in rain fed area.

Impact of SCSMV infection: The results of the field observation showed that SCSMV infection caused significant reduction on cane weight and the weight losses reached 12.17%. Meanwhile, the rendement was not considerably different between the diseased stalks and healthy stalks (Fig. 4). SCSMV infection affected the stalk height on the early growth phase but it did not influence the height in the next growth phase. Meantime, the virus infection caused remarkable reduction on stalk diameter in the early growth phase and toward harvesting. The stalk numbers per stool of diseased cane were significantly lower than healthy cane. It means that the reduction of cane weight was caused by the decrease of stalk numbers per stool and stalk diameter (Table 1).

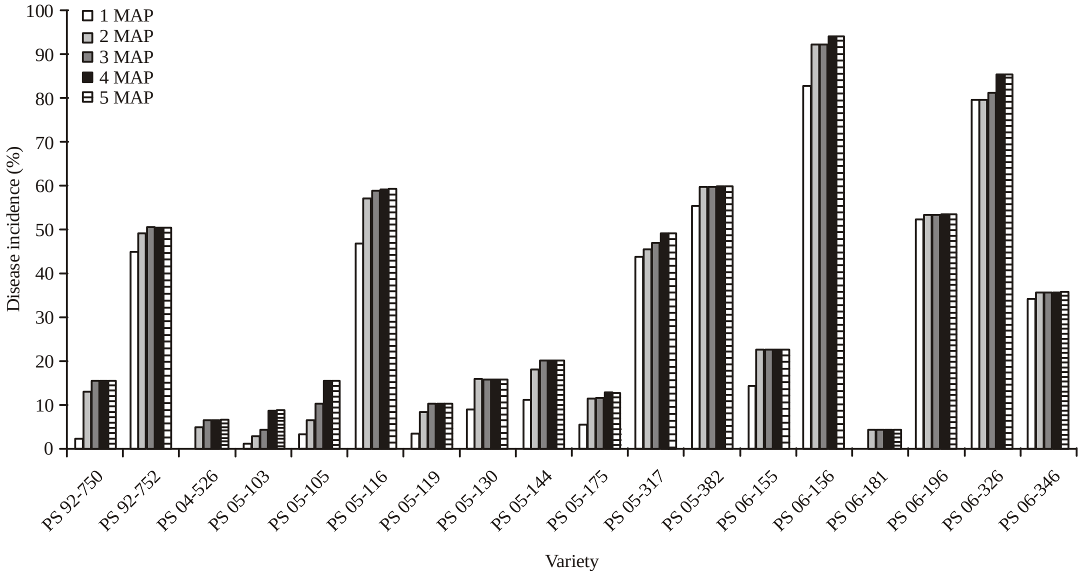

Fig. 5: Development of disease incidence of the tested varieties after mechanical inoculation

Table 1: Height, diameter and number per stool of stalk between diseased and healthy cane

Cane categories	3 MAP	6 MAP	9 MAP	12 MAP
Stalk height (cm)*				
Healthy cane	93.95^{a}	173.90^{a}	283.34^{a}	322.85^{a}
Diseased cane	84.39^{b}	157.43^{a}	264.51^{a}	305.71^{a}
Stalk diameter (cm)*				
Healthy cane	2.44^{a}	2.66^{a}	2.81^{a}	2.98^{a}
Diseased cane	2.36^{b}	2.57^{a}	2.67^{a}	2.88^{b}
∑ stalk/stool*				
Healthy cane			3.35^{a}	
Diseased cane			2.45^{b}	

*For each parameter, numbers in the same column followed by the same letter was not significantly different (α = 0.05) according to LSD test, MAP: Month after planting

Resistance of sugarcane varieties: The resistance trial showed that in general the SCSMV incidence increased gradually from 1-3 Month After Planting (MAP) and tended to stable after 3 MAP (Fig. 5). The increase of SCSMV infection from 1-2 MAP was generally higher than the increase of the infection from 2-3 MAP. Therefore, for the next resistance trial the observation of disease incidence was only done until 3 MAP because at 4 and 5 MAP the disease incidence did not increase anymore and at that time the disease symptom was technically difficult to be observed due to the plant was already quite high.

Based on the SCSMV infection rate at 3 MAP, the resistance level of the tested varieties were classified as shown in Table 2. The infection rate at 1 MAP was a critical point in determining the resistance level of the tested varieties. If the SCSMV infection rate at the early growth phase is lower, the varieties are more resistant to SCSMV. Conversely, the higher the disease incidence is more susceptible the varieties to SCSMV.

It can be seen from the Table 2, most of the promising varieties were susceptible and highly susceptible to SCSMV and the highest infection rate reached >90%. The trial revealed that only three promising varieties categorized as resistant variety namely PS 05-103, PS 06-181 and PS 04-526.

Table 2: Resistance level of the tested varieties

Varieties	Infection rate (%)	Resistance level
PS 05-103	4.6	Resistant
PS 06-181	4.8	Resistant
PS 04-526	7.0	Resistant
PS 05-105	10.5	Moderate
PS 05-119	10.7	Moderate
PS 05-175	11.8	Moderate
PS 92-750	15.8	Moderate
PS 05-130	16.2	Moderate
PS 05-144	19.7	Moderate
PS 06-155	23.2	Susceptible
PS 06-346	35.9	Susceptible
PS 05-317	47.3	Highly susceptible
PS 92-752	50.7	Highly susceptible
PS 06-196	53.7	Highly susceptible
PS 05-116	59.0	Highly susceptible
PS 05-382	60.0	Highly susceptible
PS 06-326	81.5	Highly susceptible
PS 06-156	92.3	Highly susceptible

DISCUSSION

The occurrence of SCSMV could be found at the eight locations and the virus has infected the sugarcane plantation at Jatitujuh SF and Subang SF in which SCSMV has not been reported before. This finding showed that there was an expansion of the virus distribution in Indonesia. Besides the wider distribution, the disease incidence also increased. The results of survey in 2007 found that the SCSMV incidence ranged from 0.26-62.18% (Damayanti and Putra, 2011). Meantime, in this survey we noted that the disease incidence ranged from 1.03-86.75%.

The wide occurrence of SCSMV in Indonesia is due to the transmissibility of the virus through cane cuttings and the transportation of infected cane cuttings from one area to other areas. The virus dispersal through insect vector is unknown due to there is no insect determined as a vector of SCSMV. Damayanti and Putra (2011) reported that SCSMV was transmitted vegetatively through cane cuttings and mechanically through cutting knife. Two species of aphid commonly associated with sugarcane were unable to transmit the virus namely *Rhopalosiphum maidis* (Damayanti and Putra, 2011) and *Melanaphis sacchari* (Putra, unpublished data).

Sugarcane is propagated through cane cuttings and the use of cuttings tool for preparation of planting materials is a common practice in sugarcane cultivation. In Indonesia cane growers usually do not concern about the use of disease-free plant materials. Putra *et al*. (2005, 2009) reported that cane cuttings produced by cane growers have not fulfilled the health requirement of seed cane certification. Those cane grower practices contributed to the more distribution of SCSMV in the fields.

The spread of SCSMV to Jatitujuh SF and Subang SF is an example in which the transportation of infected cane cuttings causes the more distribution of SCSMV. The survey in 2008/2009 noted that Jatitujuh SF and Subang SF were free from SCSMV. In 2009-2010 there was a shortage of seed cane in the two sugar factories and to overcome the problem they introduced cane cuttings from East and Central Java regardless the health of cane cuttings. Consequently, the infected cane cuttings were also carried to West Java and the survey in 2011 found that the virus has already infected some sugarcane fields at Jatitujuh SF and Subang SF.

The results of the survey revealed that SCSMV was more prevalent on variety PS 864. The previous survey also reported that SCSMV predominantly infected variety PS 864 (Damayanti and Putra, 2011; Putra *et al*., 2014). PS 864 was classified as highly susceptible variety

(Putra *et al.*, 2014). Therefore, it is most likely that the rapid distribution of SCSMV correlated with the dispersal of variety PS 864 in the fields. Sugiyarta (2008) reported that variety PS 864 was the dominant variety in East and Central Java. PS 864 cane cuttings mostly derived from Jombang, Mojokerto and Sidoarjo District in East Java, where the SCSMV incidences are more prevalent. Therefore, it is suspected that the infected cane cuttings of PS 864 from that district could be the source of virus distribution in Java.

Due to the widespread occurrence of SCSMV, the virus could affect sugarcane production in Indonesia. The field observation gave a preliminary indication that the virus can cause significant reduction of cane production. To control and prevent a further virus distribution the use of virus-free cane cuttings and planting resistant variety are essential. The planting susceptible variety PS 864 should be restricted especially in endemic area and has to be gradually replaced by more resistant variety. Some commercial varieties such as PS 851 and BL are resistant to SCSMV, but they are very susceptible to smut and leaf scorch disease, respectively (Putra *et al.*, 2014). Therefore, the resistant promising varieties such as PS 05-103, PS 06-181 and PS 04-526 could be projected to substitute the susceptible varieties.

CONCLUSION

From the above results, it could be concluded that there was an increase of SCSMV incidence and distribution in Indonesia. The more widespread occurrence of the disease is due to the transmissibility of SCSMV through cane cuttings and transportation of infected plant materials from one area to other areas. The rapid distribution of the virus was also correlated with the dispersal of variety PS 864. SCSMV caused a significant reduction to cane yields and also affected cane growth particularly on stalk diameter and number of stalk per stool. To control the virus the use of virus-free cane cuttings and planting resistant variety is recommended.

ACKNOWLEDGMENTS

The authors would like to thank the Indonesian Sugar Research Institute for providing financial support of this research. We also thank Mr. Herwan Cahyono Adi, Mr. Tojib, Mr. Edi Sanyoto, Mr. Mustajab, Mr. M. Sochieb and Mr. Bambang Hadiono (deceased) for excellent technical support during the research. The first author greatly acknowledges the Australian Centre for International Agricultural Research through HORT/206/147 research project for providing tuition fee for his study at Brawijaya University.

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