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RESEARCH ARTICLE

Sugarcane streak mosaic virus in Indonesia: Distribution, Characterisation, Yield Losses and Management Approaches

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Abstract Streak mosaic is a new disease of sugarcane in Indonesia caused by *Sugarcane streak mosaic virus* (SCSMV). An extensive survey conducted during milling season 2008/2009 at 30 sugar factories (SF) across the Java revealed that about 30 % of observed sugarcane fields of 28 SF were affected by the streak mosaic disease. Most commercial cane cultivars were infected by the virus but the cultivar PS 864 was found most susceptible. RT-PCR detection, using SCSMV coat protein specific gene primers SCSMV-cpF and SCSMV AP3, successfully amplified a 500 bp DNA fragment, suggesting the positive identity of the SCSMV with all the tested symptomatic samples. Protein analysis of the virus confirmed that SCSMV has a coat protein of size approximately 40 kDa and flexuous, filamentous particles about 890 nm in length was observed under an electron microscope. The virus was easily transmitted by infected cane cuttings and mechanically by sap inoculation and cutting knife. Host range test on 23 plant species revealed that maize, sorghum and *Dactyloctenium aegyptium* were alternative hosts of SCSMV. A preliminary yield loss assessment on PS 864 cultivar revealed that the disease incidence at ≥50 % reduced sugar yield by about 20 %. Hot water treatment of cane cuttings was not able to eliminate the virus in cane stalks but only postponed the appearance of the symptom. Response of 16 commercial cane cultivars to artificially inoculation of SCSMV using an abrasive pad rubbing technique showed that only five cultivars were resistant to the disease.

Keywords SCSMV · Transmission · Host range · Resistance · Yield losses · Identification

Introduction

Sugarcane is one of the important industrial crops in Indonesia, covering approximately 400,000 ha with an average yield of 60–70 tonnes of cane per ha. Sugarcane is mostly cultivated under rainfed conditions, contributing more than 60 % of the production. The Indonesian sugar industry is spread across North Sumatra, South Sumatra, Java, South and North Sulawesi. Java is still the main area for commercial sugarcane producing around 65 % of the total.

During the last 5 years, the Indonesian sugar production fluctuated and tended to decrease considerably. In 2008, sugar production in Indonesia was 2.74 million tonnes, and then in 2009 the production fell to 2.62 million tonnes (Anon 2010). In 2010, sugar production decreased again to 2.56 million tonnes (Anon 2011). There were many factors that were responsible for the decline including the presence of pests and diseases.

Recently, there was an outbreak of mosaic disease in several sugar plantations in Java Island. The disease infected some varieties that were known to be resistant to *Sugarcane mosaic virus* (SCMV) with severe mosaic symptoms. Therefore, it was suspected that a new strain of SCMV or a new virus had emerged in Indonesia. Kristini et al. (2006) reported that the mosaic symptom was caused by a new virus called *Sugarcane streak mosaic virus* (SCSMV). Since the first appearance in 2005, it is now widely distributed over commercial sugarcane in Java.

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SCSMV is a new virus reported in sugarcane and the virus has been observed in several sugar producing countries in Asia such as Pakistan, India, Thailand, Bangladesh, Sri Lanka and Vietnam (Hema et al. 2003; Chatenet et al. 2005; Viswanathan and Rao 2011). The virus is a member of a new genus in the family *Potyviridae*. The International Committee on Taxonomy of Viruses has approved *Poacevirus* as the name of the new genus, and *Triticum mosaic virus* is its prototype member (Li et al. 2011). The insect vector of the virus has not yet been reported.

Since SCSMV is a new report for sugarcane plantations in Indonesia, this paper reports the widespread occurrence of SCSMV in Indonesia and its biological characterisation, yield losses and methods of control.

Materials and Methods

Survey of SCSMV Incidence

A preliminary survey of SCSMV was carried out in 2007 at 59 cane fields of five sugar factories (SF) in Central and East Java. In the next milling season (2008/2009), an extensive survey for mapping disease distribution was conducted in 931 commercial sugarcane crops of 30 SF across Java. The mosaic symptoms were recorded during the survey and symptomatic leaves were collected and then tested using RT-PCR to determine the presence of the virus.

Virus Detection

For the microscopic observation, inoculum from sugarcane fields was transmitted on *Sorghum bicolor* cv. Rio by an abrasive pad rubbing technique (Srisink et al. 1994). The virus particles were purified using the procedure developed by Hall et al. (1998). Observations using a JEM 1010 JEOL transmission electron microscope (50,000×) was done to examine virus particles using 2 % uranyl acetate.

Protein analysis was conducted by homogenisation of the infected leaves using cracking buffer (62 mM Tris–HCl pH 6, 7.2 % SDS, 5 % 2-mercaptoethanol, 10 % glycerol, and 0.0004 % bromophenolblue) and the suspension was then heated for 2 min on 100 °C using a water-bath. Viral proteins were separated by electrophoresis in a 12.5 % polyacrylamide gel containing sodium dodecyl sulfate (SDS PAGE), and stained with coomassie brilliant blue.

For virus detection, total RNA was extracted from symptomatic leaves using RNeasy kit (Qiagen) according to manufacturer's recommendation. To confirm the presence of the virus, RT-PCR amplification using forward primer SCSMV cpF (5′-GTGGGTTCAGTTCTCGGTTC-3′) and reverse primer SCSMV-AP3′ (5,-TTTTTTCCTCCTCACG GGGCAGGTTGATTG-3′) (Putra and Damayanti 2009) was carried out.

Viral Transmission

Mechanical and Vegetative Transmission

In this glasshouse experiment, six treatments were applied for mechanical transmission of the causal virus including: (1) mechanical inoculation on spindle leaves by infected-leaf pin pricking (Sein's method); (2) mechanical inoculation on spindle leaves by an abrasive pad rubbing; (3) mechanical inoculation on younger leaves by carborundum rubbing; (4) mechanical inoculation on cane stalks using a cutting knife formerly used for cutting infected cane stalks, (5) vegetative transmission by planting SCSMV-infected cane cuttings; and (6) planting of healthy cane cuttings as a control.

For treatment 1–3, inoculation was done at 6 weeks after planting. Sap of SCSMV-infected cane leaves was used as the viral inoculum source for treatments 2 and 3. The infected leaves were blended in 0.01 M KPO_4 buffer pH 7.0 in a ratio of 1:4 w/v and the sap was filtered through cheese cloth. The inoculum was then kept in a refrigerator for 1 h. Inoculation of treatment was conducted just before planting of cane cuttings. The inoculated plants were maintained in a screen house and disease incidences were observed, based on visual symptoms until 6 months after planting. Sugarcane variety PS 864 was used as a test variety in this experiment.

Vector Transmission

Two suspected aphid species that are commonly associated with sugarcane were tested in this study namely: *Rhopalosiphum maidis* Fitch (corn aphid) and *Ceratovacuna lanigera* Zehntner (sugarcane wholly aphid). *R. maidis* was maintained on sweet corn until the 2nd generation and *C. lanigera* were collected from sugarcane variety PS 864 until the 3rd generation. Before using the insects, they were starved for 1–2 h, and then put on 2 months SCSMV-infected plants for 24 h (acquisition period). After the acquisition feeding, 25 insects were transferred to healthy sugarcane plants for 24 h (inoculation period) and then killed using an insecticide. The plants were placed in the screen house and maintained until 2 months after inoculation. Disease incidence was recorded on the basis of visual symptoms on the tested sugarcane variety.

Host Range Test

Twenty three plants of eight different families were tested for alternative host of the virus in this experiment. They

were *Gomphrena globosa, Amaranthus spinosus* (Amaranthaceae), *Chenopodium amaranticolor*, *C. quinoa* (Chenopodiaceae), *Cucumis sativus* (Cucurbitaceae), *Phaseolus vulgaris*, *Vigna unguiculata*, *Arachis hypogaea* (Leguminosae), *Lycopersicon esculentum*, *Datura stramonium*, *Nicotiana tabacum* cv. white burley, *Physalis floridana*, *Solanum melongena* (Solanaceae), and several species of the Poaceae family namely: *Sorghum bicolor, Zea mays* and common weeds of sugarcane fields in Java i.e. *Cynodon dactylon, Pennisetum purpureum*, *Cyperus rotundus, Digitaria* sp., *Dactyloctenium aegyptium, Eleusine indica* and *Echinochloa* sp.

For non-poaceae family species, the plants were mechanically inoculated by rubbing the leaves with a mixture of SCSMV inoculum in 0.01 M KPO_4 buffer pH 7.0 mixed with carborundum powder (600 mesh). However, the abrasive pad rubbing technique was used for inoculation of Poaceae family members. The inoculated plants were then maintained in the screen house under natural conditions and appearance of symptoms was recorded on the leaves, and symptomatic leaf samples were collected for RT-PCR examination.

Yield Loss Assessment

A sugarcane crop of PS 864 with different levels of SCSMV infection i.e. 0, 25, 50, 75 and 100 % was set up in an isolated field. Each treatment consisted of ten replications, each replication comprised two rows 5 m length and 20 two-eye cane cutting in each row. Level of SCSMV infection was arranged based on the proportion between healthy and diseased cane cuttings planted in each row. In 0 % infection level, all planted cane cuttings were free from SCSMV, meanwhile in 100 % infection level all the cane cuttings were infected by SCSMV. In 25, 50 and 75 % infection levels, 15 healthy two-eye cuttings and 5 diseased two-eye cuttings, 10 healthy two-eye cuttings and ten diseased two-eye cuttings, and five healthy two-eye cuttings and 15 diseased two-eye cuttings were planted, respectively. Each treatment was bordered with two rows of resistant cultivars to minimise viral transmission between the treatments. The crop was maintained with standard cultivation practices until harvesting. Disease incidence and production parameters i.e. cane tonnage, sucrose content and sugar yield were recorded.

Hot Water Treatment (HWT) Experiment

Our preliminary study revealed that the thermal inactivation point of SCSMV was 55 °C, and therefore the temperature in this experiment was set up between 52 and 55 °C. Two-eye bud cane cuttings of variety PS 864 were used to evaluate the efficacy of HWT in reducing SCSMV. The cuttings were subjected to HWT at 52, 53, 54, and 55 °C for 10, 20, and 30 min of each treatment. After treatment, the cuttings were grown in sterile soil and maintained in the screen house under natural conditions. At 2 months after planting, seed cane viability, incubation period, disease incidence and severity of symptoms were observed. Incubation period was determined based on the period between planting and the time when the mosaic symptoms first appeared on the leaf. Disease severity was assessed by estimating the percentage of leaf area with mosaic symptoms using the following scoring system: 1 = no symptoms, 2 = 0.1–5 % leaf area showing symptoms, 3 = 5.1–10 %, 4 = 10.1–20 %, 5 = 20.1–30 %, 6 = 30.1–40 %, 7 = 40.1–50 %, 8 = 50.1–75 %, 9 = 75.1–100 % (modified from Putra et al. 2003). Disease severity was counted using the following formula :

$$\mathrm{DS} = \frac{\Sigma(n_i \cdot v_i)}{N.Z} \times 100\,\%$$

where DS is the disease severity, n is the number of leaves with a certain score, v is the score, N is the number of leaves observed and Z is the highest score (9).

Resistance Trial

Sixteen commercial varieties of sugarcane i.e. PS 851, PS 862, PS 864, PS 865, PS 881, PS 882, PS 951, PSCO 902, PSBM 901, PSJT 941, Kentung, Kidang Kencana (KK), BL, GMP 1, TLH 2 and VMC 76-16. PS 864 was used as control/standard because, based on the field observation, the variety appeared more dominantly infected by SCSMV. A randomised block design with four replicates was used in this experiment. Each plot contained a 3-m row and 10 two-eye cuttings were planted in each row. A standard cultivation practice was applied during the trial.

Inoculum of SCSMV was prepared using the same procedure as described above. The abrasive pad rubbing method was used for SCSMV inoculation at 6 weeks after planting. All stalks of each variety were mechanically inoculated using the method.

Viability of cane cuttings was observed by counting the number of stools at 1 month after planting. The appearance of streak mosaic symptoms on young leaves was visually examined and leaf samples of several varieties showing the symptoms were tested using RT-PCR technique to confirm the presence of SCSMV. Disease incidence was observed 1–2 months after inoculation. Classification of resistance level adapted from resistance grading scale of SCMV namely: highly resistant with disease incidence <1 %, resistant 1–10 %, moderate 10.1–20 %, susceptible 20.1–40 %, and highly susceptible >40 % (Handojo et al. 1978).

Results

SCSMV distribution

The survey in 2007 at five SF in Central Java (Sragi SF, Madukismo SF, and Mojo SF), and East Java (Tulangan SF and KebonAgung SF) showed that mosaic symptoms similar to streak mosaic that was previously reported in other countries were observed in 38 sugarcane fields with disease incidence ranging from 0.28 to 62.18 %. The virus mostly infected commercial varieties and predominantly infected PS 864 with more severe symptoms. The typical symptoms of SCSMV were similar to those caused by SCMV. SCSMV induced systemic symptom in the form of continuous or discontinuous chlorotic streaks on sugarcane leaves and the symptom was more prominent on young leaves (Fig. 1).

The extensive survey in 2008–2009 revealed that the occurrence of SCSMV on sugarcane plantations in Java was more widespread, and 32 % of 931 observed fields were infected by SCSMV with disease incidence ranged from 0.1 to 94.7 %. The popular commercial varieties such as PS 864, BL, PS 862, and PSJT 941 were found severely infected by the virus. The disease was observed in 28 SF, and only Jatitujuh SF and Subang SF in West Java were free from SCSMV (Fig. 2).

Biological and Molecular Characterisation

Using transmission electron microscopy, filamentous flexuous particles ca. 800 nm long were observed (Fig. 3a).

Fig. 1 Typical symptoms of SCSMV on young leaves (cv. PS864)

The purified virus produced a band of ca. 40 kDa (Fig. 3b), similar in coat protein size to that reported for SCSMV by Hema et al. (1999, 2003). RT-PCR detection, using a pair of SCSMV specific coat protein gene primers SCSMV-cpF and SCSMV AP3, successfully amplified a 500 bp DNA fragment (Fig. 3c), suggesting the positive identity of the SCSMV.

Studies on transmission demonstrated that SCSMV could be transmitted mechanically through wounds made by pin pricking, carborundum, abrasive pad rubbing and cutting knife. The virus was also easily transmitted by vegetative propagation through cane cuttings. However, the virus was unable to be transmitted via insect vectors corn aphid (*R. maidis*) and sugarcane wooly aphid (*C. lanigera*) (Table 1).

The host range test revealed that the virus could infect only plants of Poaceae family viz., sorghum, maize and *Dactyloctenium aegypticum*. A systemic symptom similar to streak mosaic appeared on sorghum and maize, whereas on *D. aegyptium* there was no specific symptom but it could be detected by PCR (data not shown).

Yield Loss Assessment

The results of a preliminary yield loss assessment on variety PS 864 showed that cane tonnage and sugar yields reduced significantly at the infection level ≥50 %. The reduction ranged from 16 to 17 % and 19 to 21 % for cane tonnage and sugar yield, respectively. In contrast, SCSMV infection did not influence sucrose content (Table 2).

HWT Experiment

The HWT at 52 °C for 10 and 20 min had no effect on germination viability, while 30 min submersion time caused viability of cane cuttings to decrease upto 10 %. At 53 °C for 10 min submersion time, all tested cane setts still had 100 % viability, while 10 min longer submersion time decreased germination viability by up to 30 %. A similar trend was observed for other treatments from 54 to 55 °C in comparison with the cane setts control (Table 3). The elevation of temperature and submersion time caused cane setts to lose their viability.

The time of appearance of symptoms on leaves from setts which were subjected to HWT tended to be longer than control plants. It was revealed that increasing temperature and submersion time affected the incubation period. There was a 2–9 day delay from the appearance of the first symptoms in leaves derived from setts that have undergone HWT compared to those of control plants, although this was not statistically significant.

Severity of symptoms in all plants was significantly lower than control plants. HWT at 52 °C for 10 min

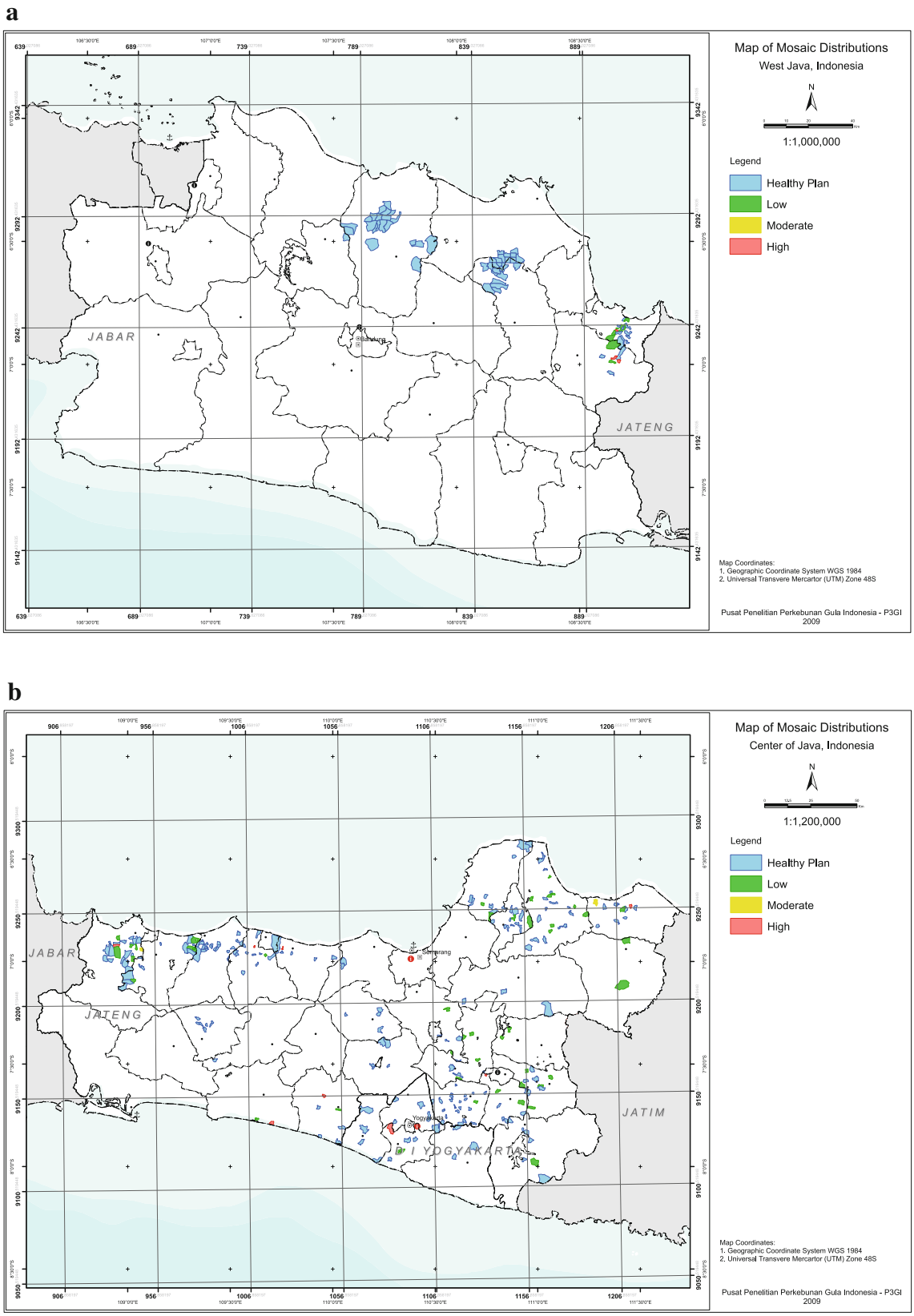

Fig. 2 SCSMV distribution across Java Island: **a** west Java; **b** central Java; **c** east Java. Note of legend: Low (disease incidence <5 %); Moderate (5–10 %); high (>10 %)

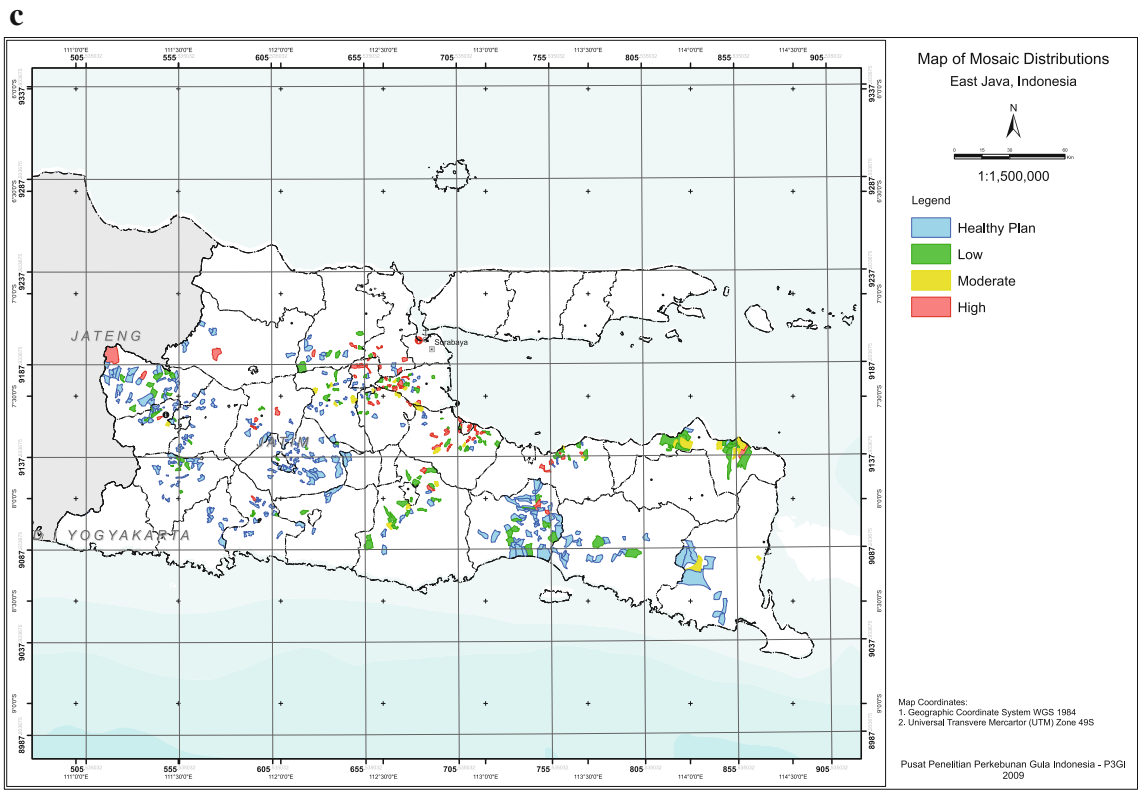

Fig. 2 continued

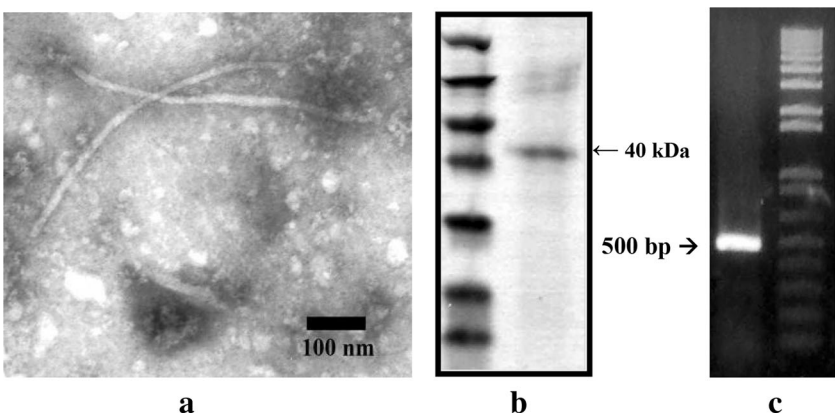

Fig. 3 **a** Transmission electron micrograph of purified virus particles of SCSMV. **b** SDS-PAGE analysis of SCSMV coat protein (40 kDa). **c** Electrophoresis of RT-PCR product of partial CP gene of SCSMV (*lane 1*), a 100 bp DNA marker (*lane 2*). *Arrow* indicates a 500-bp DNA size

showed that disease severity was significantly higher than other HWT treatments. Elevating treatment temperature and submersion time reduced the severity significantly compared to that of control plants.

Varietal Resistance

The results of the resistance trial revealed that there were 5, 6, 3 and 2 varieties classified as resistant, moderate, susceptible and highly susceptible to SCSMV, respectively (Table 4). The resistant varieties were VMC 76-16, PS 851, BL, TLH 2 and GMP 1. VMC 76-16 is a new high yielding variety released in 2010, while PS 851 is an old commercial cane which is reducing in popularity due to clonal degeneration and its susceptibility to smut. BL is a popular variety for rain-fed areas, but it is not recommended for plantations affected by leaf scorch such as at South Sumatra and Lampung due to its susceptibility to the disease. GMP 1 and TLH 2 are commercial varieties at Gunung Madu Plantation Lampung and at sugarcane plantations in Sulawesi, respectively.

Table 1 Transmission test of SCSMV

	Transmission mode	Incidence (%)	Results
A	Mechanical and vegetative transmissions		
	Mechanical transmission by Sein's method	31	+
	Mechanical transmission by abrasive pad rubbing	69	+
	Mechanical transmission by carborundum rubbing	25	+
	Mechanical transmission by cutting knife	31	+
	Vegetative transmission through infected cane cuttings	100	+
	Control (planting healthy cane cuttings)	0	−
B	Vector transmission		
	Rophalosiphum maidis	0	−
	Ceratovacuna lanigera	0	−

Note: + transmitted; − not transmitted

Table 2 Effect of SCSMV infection on cane tonnage, sucrose content and sugar yield of sugarcane cv. PS 864

Infection level (%)	Cane tonnage (t/ha)	Sucrose content (%)	Sugar yield (t/ha)
0	146.39b	7.60a	11.14b
25	124.10ab	7.45a	9.25ab
50	120.25a	7.24a	8.69a
75	122.64a	7.27a	8.95a
100	122.28a	7.32a	8.93a

Number in columns followed by the same letter are not significantly different ($\alpha = 0.05$)

Table 3 Effect of hot water treatment on cane cuttings viability, incubation period and disease severity

Temperature–time (°C–min)	Viability (%)	Incubation period (days)	Disease severity (%)
52–10	100 ± 0.00a*	16.0 ± 2.1a*	19.13 ± 5.73a*
52–20	100 ± 0.00a	16.0 ± 2.0a	15.93 ± 3.16b
52–30	90 ± 0.32ab	17.0 ± 5.7a	16.12 ± 5.80bc
53–10	100 ± 0.00a	17.0 ± 1.8a	16.00 ± 4.94bcd
53–20	70 ± 0.48abc	17.0 ± 8.4a	15.80 ± 8.31bc
53–30	70 ± 0.48abc	19.0 ± 9.2a	15.60 ± 8.42cde
54–10	70 ± 0.48abc	21.0 ± 10.1a	13.30 ± 6.80cde
54–20	60 ± 0.52abc	21.0 ± 11.0a	12.72 ± 6.70de
54–30	40 ± 0.52c	21.0 ± 10.9a	12.40 ± 6.46e
55–10	60 ± 0.52abc	23.0 ± 11.9a	11.60 ± 7.15e
55–20	50 ± 0.53bc	23.0 ± 12.2a	9.70 ± 5.60e
55–30	40 ± 0.52c	23.0 ± 11.8a	9.90 ± 5.30e
Control	100 ± 0.00a	14.0 ± 1.4a	60.00 ± 6.96f

* Number in columns followed by the same letter are not significantly different ($\alpha = 0.05$)

Table 4 Resistance level of the tested varieties

No.	Variety	Disease incidence (%)	Resistance level
1	VMC 76-16	1.4	Resistant
2	PS 851	2.6	Resistant
3	BL	3.0	Resistant
4	TLH 2	7.9	Resistant
5	GMP 1	7.9	Resistant
6	PS 882	11.7	Moderate
7	Kentung	12.2	Moderate
8	Kidang Kencana	13.3	Moderate
9	PS 862	13.5	Moderate
10	PS 951	14.9	Moderate
11	PSCO 902	19.0	Moderate
12	PSBM 901	27.5	Susceptible
13	PS 881	32.9	Susceptible
14	PS 865	35.3	Susceptible
15	PS 864	43.1	Highly susceptible
16	PSJT 941	43.8	Highly susceptible

Discussion

In Indonesia, streak mosaic is considered to be a new disease of sugarcane caused by SCSMV (Kristini et al. 2006). The incidence is widespread across Java Island and outside Java. During the extensive survey in 2008/2009, the virus was not found in the two SF in west Java i.e. Jatitujuh SF and Subang SF. However, a site observation in 2011 revealed that the virus has infected several cane fields in Jatitujuh and Subang and was also observed in Sumatra and West Papua (Putra, unpublished data).

A more widespread distribution of the virus occurred when the susceptible variety PS 864 became widely planted in Java Island and outside Java. It was suspected that the main cause of the wide distribution of the virus was due to the use of SCSMV infected cane cuttings. Being sett-borne, SCSMV spreads rapidly especially where variety PS 864 is cultivated. Further, rapid distribution of SCSMV in the fields may also be facilitated through mechanical transmission by knives during preparation of planting materials or during harvesting. Transmission through insect vectors is still questionable because no insect has been determined as a vector of the virus.

Results of this study revealed that the host range of the virus was narrow, only on members of Poaceae such as sorghum, maize and *D. aegypticum.* In Java, maize and *D. aegypticum* were commonly found growing in proximity of sugarcane plantations. These plants could serve as potential

reservoirs of SCSMV and contribute to off-season survival of the virus in the field.

Application of hot water treatment on cane cuttings could not completely eliminate SCSMV, but it could considerably reduce the disease severity. Heat affects viral replication and virus movement. It has been reported that treated plants in sustained temperature of 37 °C or above would completely inhibit multiplication of many viruses (Hadidi et al. 1998). Heat can also cause inactivation of the virus in the early phase resulting in earlier reduction in SCMV titre (Balamuralikrishnan et al. 2003). These might explain why HWT causes the incubation period to extend and reduces disease severity.

The disease causes significant reduction in cane and sugar yields. Therefore,a strategy for controlling the virus needs to be developed. Based on our study, the following are the recommended practices for effective management of the disease: (1) The use of healthy cane cuttings; (2) Planting a resistant variety such as VMC 76-16 especially in areas with high levels of SCSMV infection; (3) The development of a rapid detection method for routine detection and monitoring of the disease in the field; (4) Avoidance of intercropping between maize and sugarcane, which is a very common cultivation practice in Indonesia; (5) Use of disinfectant such as Lysol to disinfect cutting tools during preparation of planting materials at harvesting time. However, a further investigation is required for assessing the effectiveness of those recommendations on a practical scale.

Conclusions

A new mosaic disease caused by SCSMV is now widely distributed throughout Java Island and also outside Java. The virus could be easily transmitted through sugarcane setts and cutting tools. No insect has been identified as a vector of the virus. Host range of the virus is limited to the members of Poaceae family. The disease significantly reduces cane tonnage and sugar yield about 16–17 % and 19–21 %, respectively. HWT could not eliminate the virus from infected cane cuttings but delayed the appearance of the symptom and reduced disease severity. Some commercial varieties i.e. VMC 76-16, PS 851, BL, TLH 2 and GMP 1 were found resistant to SCSMV and should be planted in affected areas.

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