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Research Paper

Investigation on transmission modes and host range of *Sugarcane streak mosaic virus* in sugarcane (*Saccharum officinarum* L.) in Indonesia

Lilik Koesmihartono Putra[1,2*] • Tutung Hadi Astono[1] • Siti Rasminah Ch. Syamsidi[1] • Syamsuddin Djauhari[1]

[1]Faculty of Agriculture, Brawijaya University, Jl. Veteran Malang 65145, Indonesia.
[2]Indonesia Sugar Research Institute, Jl. Pahlawan 25 Pasuruan 67126, Indonesia.

*Corresponding author. E-mail: lilik.k.putra@gmail.com; lkputra@yahoo.com.

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Abstract. *Sugarcane streak mosaic virus* (SCSMV) is a new virus of sugarcane in Indonesia. The virus was first reported in 2005 and since then it has been widely distributed across Java Island. Since SCSMV is a new emerging virus the investigation on virus aetiology including transmission modes and virus host range is required. The results of transmission study revealed that SCSMV was transmitted through cane cuttings and caused latent infection. The virus was mechanically transmitted through cutting knife and unable to be transmitted by sugarcane aphid *Melanaphis sacchari*. The transmissibility of SCSMV through cane cuttings and cutting tools play important role in virus distribution in the field because sugarcane is propagated vegetatively using cane cuttings and cutting tools is commonly used for seed cane preparation. The host range test showed that SCSMV infected sorghum (*Sorghum bicolor*), maize (*Zea mays*), *Saccharum officinarum, Saccharum spontaneum, Saccharum robustum*, *Saccharum sinense, Saccharum barberi* and *Mischantus*. Several weed grasses namely *Brachiaria moniliformis, Panicum repens, Paspalum conjugatum*, and *Rottboelia exaltata* were also infected by the virus. Those plants are alternative hosts of the virus and could be the favourable environment for virus survival particularly when the sugarcane plants have been harvested.

Keywords: *Sugarcane streak mosaic virus*, sugarcane, transmission, host range.

INTRODUCTION

Sugarcane is one of important industrial crops in Indonesia, covering approximately 450.000 ha of land areas with an average yield of 70 tonnes of cane per ha. The sugar industry is spread across North Sumatera, South Sumatera, Java, South and North Sulawesi. Java is still the main area for commercial sugarcanes producing around 65% of total canes (Anonymous, 2010).

Sugarcane streak mosaic virus (SCSMV) is a new emerging virus infecting sugarcane in Indonesia and the virus was mainly distributed in Java. The virus was first reported in 2005 in East and Central Java (Kristini et al., 2006), and since then it has spread widely across sugarcane plantation in Java Island. An intensive survey conducted during milling season 2008/2009 revealed that SCSMV has been observed in sugarcane area of 28 sugar mills throughout Java and it was estimated that more than 30% of sugarcane areas were severely affected by SCSMV. Most of sugarcane varieties could be infected by the virus, but it was predominantly found on variety PS 864 (Kristini et al., 2008; Putra et al., 2014).

SCSMV has also been reported in several Asian countries including Bangladesh, India, Pakistan, Sri Lanka, Thailand and Vietnam. There are at least two strains of SCSMV infecting sugarcane in Asia (Chatenet et al., 2005). In India, the incidence of SCSMV in the field was more prevalent than SCMV (*Sugarcane mosaicvirus*) (Rao et al., 2006) and the virus also naturally infects

sorghum (Srinivas et al., 2010).

SCSMV is a member of genus Poacevirus in the family Potyviridae and has flexuous filamentous particles (890 × 15 nm) containing a single-stranded positive-sense RNA genome of approximately 10 kb (Hema, 2001; Xu et al., 2010; Li et al., 2011). The virus is easily transmitted through plants extract (sap) and transmitted vertically through sugarcane setts. SCSMV has narrow host range and it only infects Poaceae family like sugarcane, sorghum, maize, *Dactyloctenium aegyptium, Pennisetum glaucum, Digitaria delilis* (Hema et al., 2001; Xu et al., 2010; Damayanti and Putra, 2011; Putra et al., 2014).

No insect has been reported as a vector of the virus. Several species of aphids have been tested including *Aphis craccivora, Rhopalosiphum maidis* and *Ceratovacuna lanigera* and they are unable to transmit the virus (Hema et al., 2001; Damayanti and Putra, 2011; Putra et al., 2014). However, Viswanathan et al. (2008) reported that SCSMV could be detected using RT-PCR on sugarcane aphid colony (*Melanaphis indosacchari*). In Indonesia, *M. sacchari* is a common aphid associated with sugarcane (Kalshoven, 1981). The possibility of the aphid could transmit the virus need to be studied.

The dispersal and survival of pathogen are two important aspects of any plant disease cycle. The disease will not occur if the pathogen did not have dispersal and survival mechanism. The knowledge of dispersal and survival mechanism will reveal a weak link of the disease cycle that can be used in developing Integrated Disease Management strategy (Brown, 1997). Since SCSMV is a new emerging virus the information of virus aetiology including transmission and alternative host is very limited. The information will be useful in understanding the mechanism of virus dispersal and survival in the field. This paper reports the results of our investigation on transmission modes of SCSMV including vegetative, mechanical and insect transmission, and also host range test of the virus.

MATERIALS AND METHODS

Virus sources

Inoculum of SCSMV was collected from infected sugarcane variety PS 864 obtained from the Indonesian Sugar Research Institute Pasuruan East Java. Infected leaves or stalks 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 to 4°C for no longer than 1 h. The sap was then used immediately for the mechanical inoculation. During inoculation, the viral inoculum was placed in an icebox to maintain the infectivity of the virus.

RT-PCR detection

RT-PCR tests were applied in this study to confirm the presence of SCSMV in sugarcane leaf samples. Total RNA was extracted from the leaf samples using single direct tube method adapted from (Suehiro et al., 2005). To detect the presence of the virus, RT-PCR using forward primer SCSMV cpF (50-GTGGGTTCAGTTCTCGGTTC-30) and reverse primer SCSMV-AP30 (5,-TTTTTTCCTCCTCACGGGGCAGGTTGATTG-30) (Damayanti and Putra, 2011) was carried out to amplify a 500 bp DNA fragment of partial coat protein gene (CP) and the 3' terminal of SCSMV. PCR condition was 35 cycles at 94°C for 30 s, 47°C for 1 min, and 72°C for 2 min and a final extension on 72°C for 10 min.

Transmission test

Vegetative transmission

To investigate whether SCSMV could be vegetatively transmitted through cane cuttings, three sources of cane cuttings were compared in this experiment namely: 1) cane cuttings obtained from SCSMV-infected plants; 2) cane cuttings of symptomless plant obtained from plantation affected by SCSMV; and 3) healthy cane cuttings. Three cane varieties, that is, PS 862, PS 864 and VMC 76-16 were used in this trial.

Single-eye cane setts were planted in 25-cm-diameter polybags of planting media containing the mixture of soil, sand and compost (2:1:1) with the eye facing up and covered lightly with the mix. The polybags were placed in a glasshouse and arranged in a randomized block design with three replications, three sources of cane cuttings as blocks and each treatment unit contained six polybags. Disease incidences were observed at 1, 2 and 3 months after planting. RT-PCR technique was applied to confirm the presence of the virus on leaf samples showed mosaic symptoms.

Mechanical transmission

To examine whether SCSMV could be mechanically transmitted, five treatments were applied for the test including: 1) mechanical transmission on cane stalks by a cutting knife and sap of SCSMV-infected leaves as viral inoculum; 2) mechanical transmission on cane stalks by a cutting knife and sap of SCSMV-infected stalks as viral inoculum; 3) mechanical transmission by an abrasive pad rubbing and sap of SCSMV-infected leaves as viral inoculum; 4) mechanical transmission by an abrasive pad rubbing and sap of SCSMV-infected stalks as viral inoculum; and 5) planting of healthy cane cuttings as a control.

The cutting knife inoculation was carried out just before planting by dipping the knife in the viral inoculum for 2 to 3 s and then the knife was used to cut healthy cane stalks into single-eye cane cuttings. The inoculated cane

cuttings were planted in 25-cm-diameter polybags of the planting media. For other treatments, healthy single-eye cane cuttings were planted in the polybags and the plants were inoculated 6 weeks after planting using an abrasive pad rubbing technique (Srisink et al., 1994), except for control treatment. The abrasive pad inoculation was conducted by pulling a folded inoculum-soaked Scotch-Brite® abrasive pad along the spindle leaves. The inoculated plants were maintained in a screen house and the polybags were arranged in a randomized complete design with 3 replicates. Disease incidences were observed based on visual symptoms until 4 months after planting. Sugarcane variety PS 864 was used as a test variety in this experiment.

Insect transmission

Insect transmission test was performed with *Melanaphis sacchari* (Zehntner), an aphid species which commonly colonize sugarcane in Indonesia (Kalshoven, 1981), to determine whether SCSMV could be transmitted by the aphid species from symptomatic to healthy sugarcane variety PS 864. The aphids were collected from fields at Indonesian Sugar Research Institute and reared on healthy sugarcane plants variety PS 864. The first generation of the reared aphids was used in this experiment and 5 rates of aphid number were examined namely: 5, 10, 15, 20 and 25. The aphids were starved for 1 h in a petri dish and then placed on the SCSMV-infected sugarcane allowing a virus acquisition feeding periods for 24 h. For inoculation feeding, aphids were transferred to healthy sugarcane for 24 h. Test plants were covered by insect cages (30 × 30 × 100 cm) during the inoculation period and then the aphids were killed by spraying using insecticide.

The experiment was carried out in the insect free glass house and the tested plants were arranged in a randomized complete design with 3 replicates. Healthy sugarcane plants not exposed to aphids were placed with the tested plants to serve as control. Observation for symptom was conducted for 1 to 2 months after inoculation and the presence of the virus was detected using RT-PCR.

Host range test

To determine the alternative host of SCSMV that could become the place for virus survival or initial virus inoculum in the fields, sixteen species of Poaceae family were tested in the experiment. They were three kinds of cultivated plants including sorghum (*Sorghum bicolor* cv. Rio), maize (*Zea mays*) and rice (*Oryza sativa*); wild canes and their relatives including *Saccharum officinarum, Saccharum spontaneum, Saccharum robustum, Saccharum sinense, Saccharum barberi, Erianthus,* and *Miscanthus*; and weed grasses of sugarcane plantation in Indonesia including *Brachiaria moniliformis, Chloris barbata, Imperata cylindrica, Panicum repens, Paspalum conjugatum* and *Rottboelia exaltata.* The tested plants were established in 25-cm-diameter polybags of the planting media with different replication numbers depend on the availability of the plants. The tested plants were mechanically inoculated with SCSMV using abrasive pad rubbing technique (Srisink et al., 1994). Observation of symptom was conducted at 1 to 2 months after inoculation and the presence of the virus was detected using RT-PCR.

RESULTS

Transmission test

Vegetative transmission

The results of the vegetative transmission test (Figure 1) revealed that the symptomatic cane cuttings of the three varieties produced plants with mosaic symptoms. The disease incidences varied among the three varieties. No mosaic symptom was observed on cane cuttings of symptomless plants of variety PS 864 and VMC 76-16, meanwhile asymptomatic cane cuttings of variety PS 862 produced mosaic symptom 2 months after planting. At 1 month after planting of diseased cane cuttings the disease incidence of variety PS 864 and VMC 76-16 reached 72.22% and these values were significantly higher than PS 862, meanwhile for healthy and symptomless cane cuttings there were no significant differences among the three varieties (Table 1). Until 3 months after planting more than 80% plants from symptomatic cane cuttings showed mosaic symptoms and this was considerably higher than symptomless and healthy cane cuttings (Table 2). At 2 to 3 months after planting among the three varieties the disease incidence of variety PS 862 reached 40.74% and it was significantly higher than other two varieties (Table 3).

From this experiment it was proved that SCSMV not only can be transmitted vegetatively through cane cuttings but also can cause latent infection. The planting of diseased cane cuttings and symptomless cane stalks derived from SCSMV-infected cane nursery will obviously produce diseased canes.

Mechanical transmission

There was a different pattern of the diseases development between cutting knife and abrasive pad treatment (Figure 2). In the cutting knife treatments disease incidences at 1 to 3 months after planting were relatively stable and then gradually increased at 4 month after planting. Meanwhile, in abrasive pad treatments the

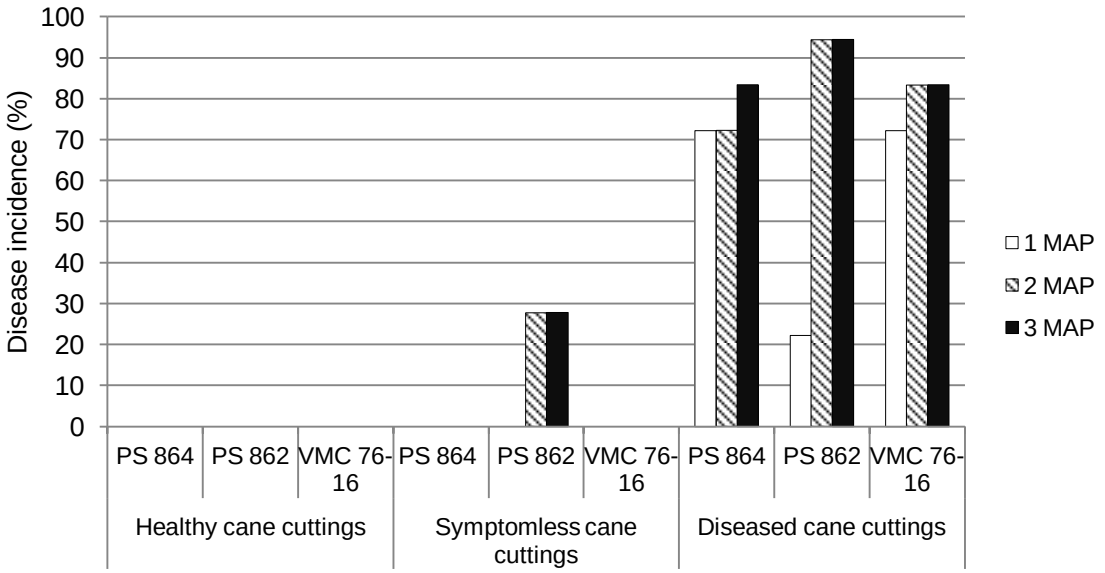

Figure 1. Disease incidences of the three sources of cane cuttings on three different sugarcane varieties. MAP = month after planting.

Table 1. Disease incidences of three sources of cane cuttings from three different varieties at 1 month after planting.

Variety	Disease incidence (%)[a]		
	Healthy cane cuttings	Symptomless cane cuttings	Diseased cane cuttings
PS 862	0[a]	0[a]	22.22[b]
PS 864	0[a]	0[a]	72.22[a]
VMC 76-16	0[a]	0[a]	72.22[a]

[a] Numbers in the same column followed by the same letter was not significantly different (α = 0.05) according to LSD test.

Table 2. Disease incidence of three sources of cane cuttings at 2 and 3 months after planting.

Cane cutting sources	Disease incidence (%)[a]	
	2 months after planting	3 months after planting
Healthy cane cuttings	0[b]	0[b]
Symptomless cane cuttings	9.26[b]	9.26[b]
Diseased cane cuttings	83.33[a]	87.04[a]

[a] Numbers in the same column followed by the same letter was not significantly different (α = 0.05) according to LSD test

disease incidences increased at the beginning and then tended to stable at 3 months after planting.

The results of mechanical inoculation test showed that SCSMV can be transmitted through mechanical wound on stalks and leaves caused by cutting knife and abrasive pad respectively. Sap of infected leaves and infected stalks could be the sources of viral inoculum for mechanical inoculation. In general the disease incidence of abrasive pad inoculation using infected leaf sap and the cutting knife inoculation using infected stalk were significantly higher than un-inoculated treatment. No considerable difference between the abrasive pad treatment using infected leaf sap and the cutting knife treatment using infected stalk sap (Table 4). This result revealed that the virus transmission through cutting knife was as effective as mechanical transmission using abrasive pad rubbing method.

Insect transmission

Insect transmission test using *Melanaphis sacchari* did

Table 3. Disease incidences of three sources of cane cuttings at 2 and 3 months after planting.

Varieties	Disease incidence (%)[a]	
	2 months after planting	3 months after planting
PS 862	40.74[a]	40.74[a]
PS 864	24.07[b]	27.78[b]
VMC 76-16	27.78[b]	27.78[b]

[a] Numbers in the same column followed by the same letter was not significantly different (α = 0.05) according to LSD test.

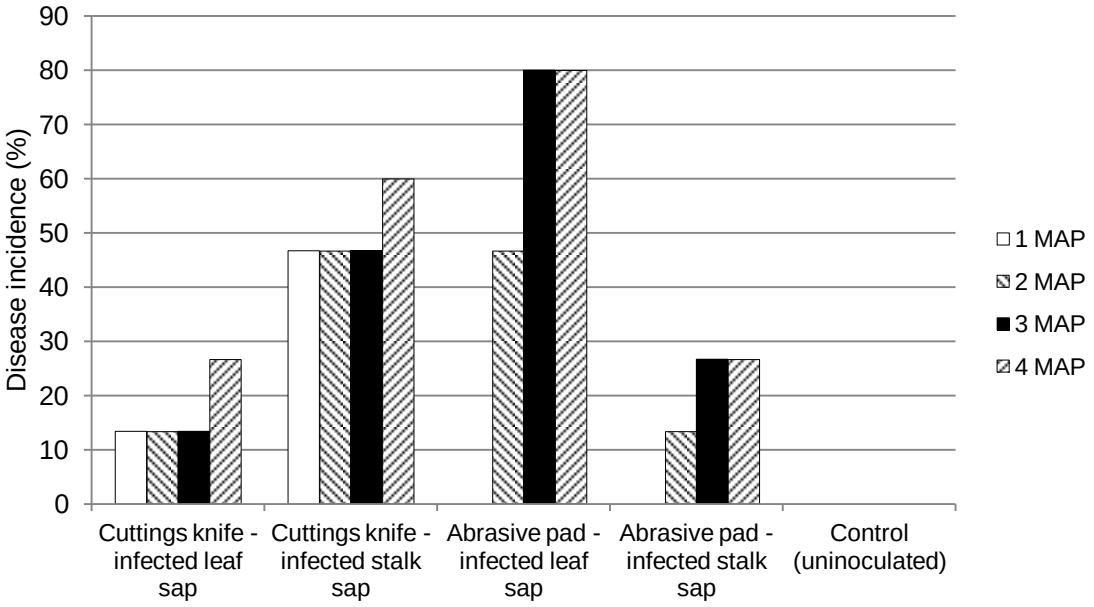

Figure 2. Disease incidences of all mechanical inoculation treatments. MAP = month after planting.

Table 4. Disease incidences of all mechanical inoculation treatments.

Treatments	Disease incidence (%)[a]		
	2 MAP[b]	3 MAP[b]	4 MAP[b]
A. Cutting knife - infected leaf sap	13.33[ab]	13.33[b]	26.67[bc]
B. Cutting knife - infected stalk sap	46.67[a]	46.67[ab]	60.00[ab]
C. Abrasive pad - infected leaf sap	46.67[a]	80.00[a]	80.00[a]
D. Abrasive pad - infected stalk sap	13.33[ab]	26.67[b]	26.67[bc]
E. Control (uninoculated)	0[b]	0[b]	0[c]

[a] Numbers in the same column followed by the same letter was not significantly different (α= 0.05) according to LSD test.
[b] MAP = month after planting.

not give positive results. All treatments with different rate of aphid population did not produce mosaic symptoms on the tested plants and no virus was detected by RT-PCR on leaves samples (Figure 3). The results indicated that *M. sacchari* was unable to transmit the virus. Therefore, the insect is not a vector of SCSMV. Xu et al. (2010) stated that due to the absence of KITC, PTK, and DAG motifs in the HC-Pro and CP of SCSMV, the virus is probably not transmitted by aphids.

Host range test

The results of host range study showed that most of the tested plants had mosaic symptoms (Table 5). In the cultivated plants SCSMV infected sorghum and maize but

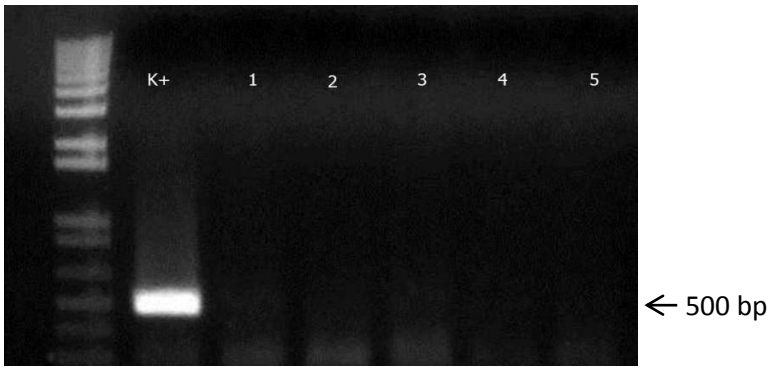

Figure 3. Gel electrophoresis of RT-PCR of the insect transmission test. (K+) positive control; 1) 5 insects/plant; 2) 10 insects/plant; 3) 15 insects/plant; 4) 20 insects/plant; 5) 25 insects/plant.

Table 5. The results of SCSMV host range test on several species of Poaceae family.

Plant species	∑ infected plants / ∑ total inoculated plants	Incubation period (days)	Symptoms	Results of RT-PCR
Cultivated plants				
1. *Sorghum bicolor* cv. Rio	20 / 20	12 - 20	Mosaic	+
2. *Oryza sativa*	0 / 10	-	-	−
3. *Zea mays*	4 / 10	21 - 30	Mosaic	+
Wild canes and their relatives				
1. *Saccharum officinarum*	4 / 10	16 - 24	Mosaic	+
2. *Saccharum robustum*	16 / 23	16 – 26	Mosaic	+
3. *Saccharum spontaneum*	3 / 15	18 - 30	Mosaic	+
4. *Saccharum sinense*	7 / 7	20 – 23	Mosaic	+
5. *Saccharum barberi*	10 / 14	18 - 30	Mosaic	+
6. *Erianthus*	0 / 25	-	-	−
7. *Miscanthus*	9 / 11	16 - 19	Mosaic	+
Weed grasses				
1. *Rottboelia exaltata*	8 / 12	12-16	Mosaic	+
2. *Paspalum conjugatum*	1 / 12	16	Mild mosaic	+
3. *Panicum repens*	1 /12	16	Mild mosaic	+
4. *Brachiaria moniliformis*	1 /12	14	Mild mosaic	+
5. *Imperata cylindrica*	0 / 12	-	-	−
6. *Chloris barbata*	0 / 12	-	-	−

not for rice. For sorghum 100% of inoculated plants showed mosaic symptoms and it indicated that *Sorghum bicolor* cv Rio is susceptible to SCSMV. All wild cane species and their relatives were infected by SCSMV, except *Erianthus*. Several weeds commonly found in sugarcane plantation also were infected by SCSSMV including *Rottboelia exaltata, Brachiaria moniliformis, Panicum repens* and *Paspalum conjugatum.* Meanwhile, *Imperata cylindrica dan Chloris barbata* gave asymptomatic results. The high level of infection rate occurred on *S. bicolor* cv. Rio, *S. robustum, S. sinense, S. barberi*, *Miscanthus* and *R. exaltata.*

Most of the infected plant showed clearly mosaic symptoms in the form of continuous or discontinuous chlorotic streaks on the leaves, except on *B. moniliformis, P. repens* and *P. conjugate* which exhibited mild mosaic symptoms. The symptoms were generally more obvious on younger leaves. The results of RT-PCR test revealed that the virus could be detected on the leaf sample of the infected plants and the mild mosaic symptoms of *B. moniliformis, P. repens* and *P. conjugate* produced smear bands (Figure 4). Those plants with positive results are

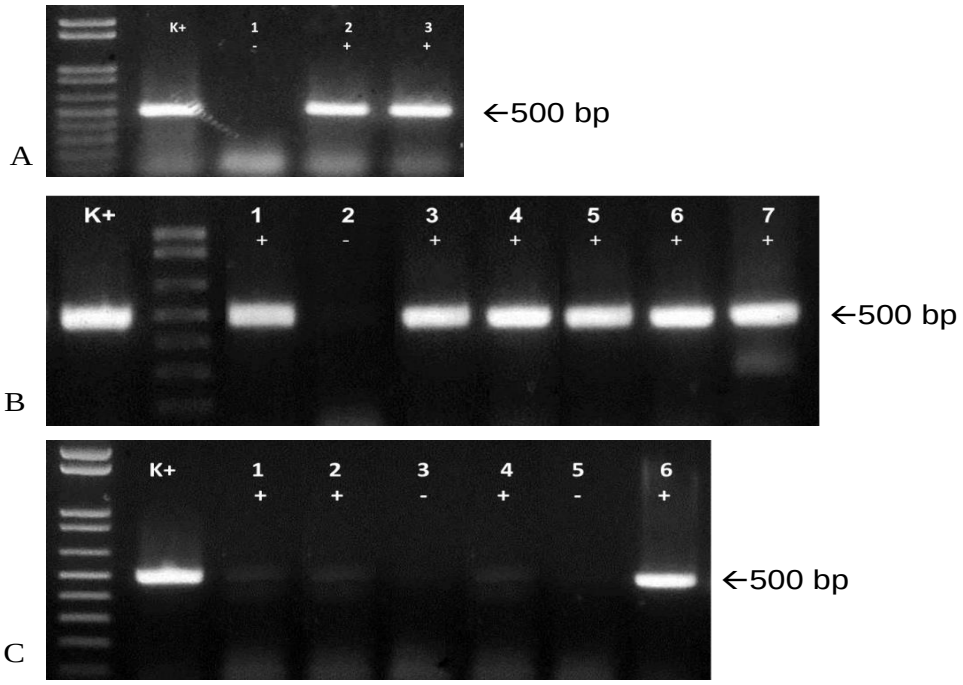

Figure 4. Gel electrophoresis of RT-PCR of the host range test. (A) Cultivated plants: (K+) positive control; (1) *Oryza sativa*; (2) *Zea mays*; (3) *Sorghum bicolor*. (B) Wild canes and their relatives: (1) *Mischantus*; (2) *Erianthus*; (3) Saccharum officinarum; (4) *Saccharum robustum*; (5) *Saccharum spontaneum*; (6) *Saccharum sinensis*; (7) *Saccharum berberi*; (K+) positive control. (C) Weed grasses: (K+) positive control; (1) *Paspalum conjugatum*; (2) *Panicum repens*; (3) *Cloris barbata*; (4*) Brachiaria moniliformis*; (5) *Imperata cylindrical*; (6) *Rottboelia exaltata.*

alternative hosts of SCSMV.

DISCUSSION

The results of transmission study revealed that SCSMV was vegetatively transmitted through cane cuttings and it can cause latent infection. The same finding was also reported by Hema et al. (2001), Damayanti and Putra (2011), and Putra et al. (2014). Agrios (2005) explained that the vegetative transmission occurs when the virus cause systemic infection. In such infection the virus not only confine in the site of primary infection but also spread to other parts of plant. The plants with systemic infection will contain the virus during their life because the plants have no mechanism to eliminate the virus. Therefore, any parts of the infected plant used for vegetative propagation will contain the virus. SCSMV is a virus that causes systemic infection on sugarcane and sugarcane is propagated using cane stalk. Consequently, the virus is vertically transmitted through vegetative propagation. Cane cuttings derived from diseased plants will generate infected stools. Due to SCSMV can also cause latent infection, asymptomatic cane cuttings obtained from infected plants could produce diseased canes. Planting of infected cane cuttings will cause the disease incidence more prevalent in the field and also distribute the virus to a new sugarcane plantation that is still free from the virus.

The results of the transmission tests also showed that SCSMV was mechanically transmitted through cutting knife and *M. sacchari* was to unable to transmit the virus. So far no insect has been reported as a vector of SCSMV. Therefore, transmission through cane cuttings and cutting knife could be an effective way for SCSMV distribution in the fields because sugarcane is propagated using cane cuttings and cutting knife is commonly used by cane growers for preparation of planting materials and also for harvesting. To prevent the more spread of SCSMV, the use of virus-free plant materials and the application of disinfectant for sterilized the knife or other cultivation equipment are certainly recommended.

The host range test using mechanical inoculation indicated that beside sugarcane SCSMV also infects other cultivated plants like sorghum (*Sorghum bicolor*) and maize (*Zea mays*). The same result was also reported by Hema et al. (2001), Xu et al. (2010), Damayanti and Putra (2011), and Putra et al. (2014). In India SCSMV has been reported to naturally infect the sorghum crops grown nearby or some distance away from sugarcane fields (Srinivas et al., 2010). *Sorghum bicolor* cv. Rio is very susceptible and therefore it is a

suitable host for virus propagation.

In this experiment, it was found that all tested wild canes and their relatives (*Saccharum* complex) showed streak mosaic symptoms, except *Erianthus*. This is the first report that SCSMV could infect most of *Saccharum* complex. It seems that *Erianthus* has a high resistance to SCSMV and therefore it could be considered as resistance gen sources of SCSMV for sugarcane breeding program. Hybridization of *Erianthus* with sugarcane improved resistance against mosaic disease caused by SCMV (Grisham, 1992), smut disease caused by *Ustilago scitaminea* (Burner, 1993; Shen, et al., 2014) and red rot disease caused by *Colletotricum falcatum* (Ram et al., 2001).

Several weed grasses also proved to be mechanically infected by SCSMV such as *Rottboelia exaltata, Brachiaria moniliformis, Panicum repens* and *Paspalum conjugatum. Rottboelia exaltata* seems to be susceptible against SCSMV indicated by the high level of infection (67%), meanwhile the three other weed grasses could be carrier of the virus. Other weed grasses that have been reported to be infected by SCSMV were Sudan grass, Johnson grass and *Digitaria delilis* (Hema et al., 1999; Xu et al., 2010) and *Dactylactonium aegypticum* (Damayanti and Putra, 2011; Putra et al., 2014).

Those plants positively infected by SCSMV are alternative hosts of the virus. In Indonesia maize is frequently planted close by sugarcane crops and weed grasses such as *R. exaltata*, *B. moniliformis, P. repens*, *P. conjugatum* and *D. aegypticum* are commonly found in sugarcane plantation. The presence of the alternative hosts in the fields could be the place for off-season survival of the virus and the source of viral transmission. Therefore, avoidance of planting maize near sugarcane crops and control of those weeds are recommended as part controlling or managing the disease.

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