Jurnal Perlindungan Tanaman Indonesia, Vol. 26, No. 2, 2022: 148–158
DOI: 10.22146/jpti.71158
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Sugarcane streak mosaic virus (ScSMV) Resistance Evaluation of Sugarcane Varieties

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Received December 27, 2021; revised January 16, 2022; accepted December 20, 2022

ABSTRACT

Sugarcane streak mosaic virus (ScSMV) is the most important vi ral disease of sugarcane in Indonesia with distribution in almost all commercial sugarcane plantations. The disease causes significant yield losses of both cane tonnage and sugar yield. The use of resistant varieties is the best approach for controlling viral diseases. This study aims to investigate resistance response of several introduced varieties against ScSMV in a glasshouse condition and the impact of the viral infection on chlorophyll and proline content in sugarcane leaves. Sugarcane plants were inoculated using ScSMV inoculum one month after planting using an abrasive pad rubbing method. Disease incidence and severity was observed at week 4–12 after inoculation and variety resistance levels were classified based on disease incidence. Confirmation of the virus was done by RT-PCR. Spectrophotometer was used to measure chlorophyll content at dual wavelengths of 645 and 663 nm, and proline content at wavelengths of 520 nm. The results showed that most of the tested varieties were susceptible to ScSMV. There are six highly resistant varieties, namely SRA 1, SRA 2, N 10-4, N 10-7, N 10-9, and N 10-13, but these varieties still require to be tested on a field scale. ScSMV infection generally decrease chlorophyll and proline content. However, the physiological effect of ScSMV infection on chlorophyll and proline content needs further investigation.

Keywords: sugarcane; *Sugarcane streak mosaic virus* (ScSMV); varietal resistance

INTRODUCTION

Sugarcane streak mosaic virus (ScSMV) is the most prevalent and rapidly spreading sugarcane disease in Indonesia. The presence of ScSMV was first reported in 2005 in East and Central Java (Kristini *et al*., 2006), and since then the virus has spread rapidly to other sugarcane areas infecting commercial variety, such as PS 862, PS 864, BL, PS 881, PS 951, PSJT 941, and Kidang Kencana, and severe incidences occurred in the highly susceptible varieties, namely PS 864 and PS 881 (Putra *et al*., 2014; Putra *et al*., 2015). The virus is now widely distributed in most of sugarcane plantations both in Java and outside Java (Putra *et al*., 2014; Margarey *et al*., 2018; Putra, *et al*., 2022).

Putra *et al*. (2014) stated that the rapid and wide distribution of ScSMV was mainly due to the use of ScMV-infected cane cutting and widespread planting of the highly susceptible varieties, namely PS 864 and PS 881. ScSMV is transmitted by planting materials causing virus dispersal outside Java were related to the use of cane cuttings originating from Java (Margarey *et al*., 2018).

ScSMV can cause significant sugarcane yield losses. Field observations showed preliminary indication that ScSMV infection cause reduction in cane

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production of about 12.17% (Putra *et al*., 2015). Yield losses assessment on PS 864 revealed that at infection rate of more than 50%, reduced cane tonnage and sugar yield ranging 16–17% and 19–21%, respectively (Putra *et al*., 2014).

Putra *et al*. (2014) recommended strategy for controlling streak mosaic disease was by implementing integrated disease management, and the main approaches was the use of healthy cane cuttings and planting resistant varieties. ScSMV is unable to be completely eliminated by hot water treatment on infected cane cuttings (Damayanti *et al*., 2010) and the application of a combination of several endophytic bacteria is also unable to suppress disease incidence (Damayanti *et al*., 2011). Therefore, replacing highly susceptible commercial varieties, such as PS 864 and PS 881, with more resistant varieties should be a priority. Substitute varieties can be obtained from sugarcane variety of domestic breeding programs or introduced varieties from abroad. The objective of this study is to determine the resistance level of 23 introduced varieties against ScSMV. The resistant varieties determined in this study could potentially be used for controlling ScSMV after undertaking field adaptation tests or as parental in sugarcane breeding programs.

MATERIALS AND METHODS

This research was conducted in the glasshouse of the Indonesian Sugar Research Institute (ISRI), Pasuruan during December 2018–August 2019. The experimental was arranged in a Completely Randomized Design (CRD) with four replicates. There were 23 introduced sugarcane varieties tested in this study consisting of 10 varieties from Australia and 13 varieties from Brazil. The standard or control varieties used in this trial were GMP 7 for resistant variety, PS 091 for moderate resistant variety, and PS 864 and PS 881 for susceptible varieties. For each variety, two healthy single-eye cane cuttings were planted in 30-cm-diameter plastic pots containing a mixture of soil, sand and compost (2:1:1) with the eye facing up and covered lightly with the mix. All plant materials of sugarcane varieties used in this study were derived from tissue culture propagation and grown in a greenhouse to minimize the occurrence of unintended viral infections from other sources.

Preparation of ScSMV Inoculum

ScSMV inoculum was obtained from infected variety PS 864 at the Pathology collection farm of ISRI. The stem sap of infected sugarcane was used as inoculum sources because it is more infective than the sap of infected leaves (ISRI, unpublish document,). The infected sugarcane plant was tested by PCR to ensure that the inoculum was a valid source of ScSMV.

The 6-month-old infected cane stems were cut down, and 200 grams of the stems were blended by adding 1 liter of buffer phosphate pH 7. The juice was filtered through a cheesecloth and put into a sterile bottle. The filtered sap was then stored in a refrigerator at 5ºC for 1 hour. The sap was then used for mechanical inoculation and during inoculation, the viral inoculum was chilled on ice.

ScSMV Inoculation

ScSMV inoculation to sugarcane plants was carried out one month after planting using an abrasive pad rubbing method (Srisink *et al*., 1994). The abrasive pad was dipped into the ScSMV inoculum and then rubbed on the leaves of the tested plants by pulling the leaf between the pads.

Disease Observation

Symptoms were observed every day after inoculation until the first symptoms appeared to determine incubation period. Disease incidence and diseases severity were carried out every week by observing the symptoms and counting infected plants starting from week 1 until week 12 after inoculation. To confirm the observed mosaic symptoms were infected by ScSMV, several leaf samples from symptomatic plants were examined by RT-PCR.

Diseases incidence. Diseases incidence was counted using the following formula (Abadi, 2000):

$$DI = \frac{n}{N} \times 100\%$$

DI is the diseases incidence, n is the number of infected plants, N is the number of plants tested.

Diseases severity. Disease severity was assessed using scoring system adapted from Putra (2015). The assessment was done by estimating the percentage of leaf area with mosaic symptoms on spindle leaves, top three exposed leaves (the first to the third visible

dewlap leaves), and tillers. The scoring system use the following categories: 0= no symptom; 1 = fine/thin mosaic pattern on spindle and first visible dewlap leaves, mosaic symptoms covering ≤20% of the symptomatic leaf surface; 2 = fine/thin mosaic pattern on spindle and first visible dewlap leaves, mosaic symptoms covering >20–50% of the symptomatic leaf surface; 3 = clear mosaic pattern on spindle and top three exposed leaves, mosaic symptoms covering ≤50% of the symptomatic leaf surface; 4 = clear mosaic pattern on spindle and top three exposed leaves, mosaic symptoms covering > 50% of the symptomatic leaf surface, healthy tillers; 5 = clear mosaic pattern on spindle and top three exposed leaves, mosaic symptoms covering ≤50% of the symptomatic leaf surface, partially infected tillers; 6 = the mosaic pattern is obvious on both spindle leaves and top three exposed leaves, mosaic symptoms covering > 50% of the symptomatic leaf surface, all the tillers are infected; 7= the mosaic pattern and chlorosis are obvious on either spindle or top three exposed leaves, mosaic symptoms covering ≥50% of the symptomatic leaf surface, all tillers are infected with severe chlorosis, retarded plant growth. Disease severity was counted using the following formula:

$$DS = \frac{\Sigma(n \times v)}{N \times z} \times 100\%$$

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.

Plant Resistance Category

The resistance level to ScSMV was determined based on disease incidence using score/class method referred to Legowo (1993) as follows: 0% = Highly resistant, 0.1–5% = Resistant, 5.1–10% = Moderate resistant, 10.1–40% = Susceptible, > 40% = Highly susceptible.

ScSMV Detection Using RT-PCR (Reverse Transcription-Polymerase Chain Reaction)

Total RNA was extracted from symptomatic leaves using RNeasy kit (Qiagen) according to manufacturer's recommendation. Total RNA (3 ul) was used as a template for cDNA construction and then RT-PCR amplification was carried out. cDNA (1 ul) was mixed into cocktail PCR (2.5 ul 10× PCR buffer, 0.5 ul 210 mM dNTP, 1 ul of forward and reverse primers each (10 uM), 2.5 U of Taq polymerase, sterile H_2O up to a total premix PCR of 25 ul). The forward primer ScSMV-cpF (5'-GTGGGTTCAGT TCTCGGTTCGTAGC-3') (Damayanti & Putra, 2011) and the reverse primer ScSMV-AP3' (5'-TTT TTTCCTCCT CACGGGGCAGGTTGATTG-3') (Hema *et al.*, 2003) was used to amplify a 500 bp DNA fragment of partial coat protein gene (CP) and the 3' terminal of ScSMV. RT-PCR was performed 35 cycles at 94°C for 30 seconds, 47°C for 1 minute, and 72°C for 2 minutes and final extension at 72°C for 10 minutes at 4°C.

PCR products were separated by agarose gel electrophoresis using 1.5% gel agarose in Tris/Borate/EDTA (TBE) containing ethidium bromide (0.5 ug/ml) for 15 minutes on 100 Volt. DNA visualization was done under a UV illuminator and was documented by using a digital camera.

DNA Sequencing and Phylogenetic Tree Construction

PCR product of samples with positive results was sent to PT Genetics Science Jakarta for nucleotide sequencing. The sequencing results were used to find homologous DNA sequences in the database (GenBank) using the Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnology Information (NCBI). Phylogenetic tree was constructed using BioEdit and Mega 10 software using the neighbour-joining method.

Observation of Chlorophyll Content

Chlorophyll content was determined three months after inoculation by taking the lamina of the first visible dewlap (FDV) leaf. For each sample, 1 g of the leaves and 20 ml of 80% acetone were crushed in mortar and filtered using filter paper. The filtrate was collected and put into a cuvette to measure chlorophyll content on spectrophotometer with absorbance 645 nm and 663 nm. Chlorophyll levels was calculated according to the Arnon method (1949), using formula: chlorophyll-a = (12.7×A663) – (2.69×A645) mg chlorophyll/g and chlorophyll b = (22.9×A645) – (4.68 ×A663) mg of chlorophyll/g total chlorophyll = 20.2×A645 + 8.02×2.640

Observation of Proline Content

Proline content was measured three months after inoculation by taking the lamina of the FDV leaf. For each sample, 0.1 g of the leaf samples with 2 ml

of 3% sulphosalicylic solution were ground in a mortar and then filtered through Whatman filter paper no. 40. Filtrate 2 ml was reacted with 2 ml of ninhydrin acid and 2 ml of 100% glacial acetic acid in a test tube, then heated at 100°C for 1 hour. The reaction was ended by chilling into ice for 15–20 minutes. The mixture of filtrate solution was then added with 5 ml of toluene and stirred for 15–20 seconds until two layers of different colored liquid were formed. Red toluene containing proline was pipette and then put into a cuvette to measure the proline content using a spectrophotometer at 520 nm with a toluene as blank (Bates *et al.*, 1973).

Data Analysis

Data were analysed using SPSS 16.1. If the ANOVA results were significantly different, the data were then tested by the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Incubation Period

The incubation period of streak mosaic disease on the tested varieties ranged from 7–34 days after inoculation. N 10-5 variety had the fastest incubation period of 7 days after inoculation, while N 10-2 variety had the longest incubation period of 34 days after inoculation. Several varieties did not show streak mosaic symptoms, namely SRA 1, SRA 2, N 10-4, N 10-7, N 10-9, N 10-13, and GMP 7 (Figure 1).

Visual Symptoms of ScSMV

The plants were successfully inoculated with ScSMV indicated by the appearance of mosaic symptoms on sugarcane leaves in the form of short greenish-yellow chlorotic stripes. Symptoms of streak mosaic varied in each variety and the symptom was more prominent on young leaves. There were four different phenotypes of streak mosaic symptoms (Figure 2), namely: a) short chlorotic lines spread on the leaf surface; b) short chlorotic lines that were closed together and almost covered the entire leaf surface; c) mild chlorotic lines almost covered the entire leaf surface; and d) slightly elongated and very clear chlorotic lines.

Those symptoms of streak mosaic were similar to the report of Putra *et al.* (2014), which described

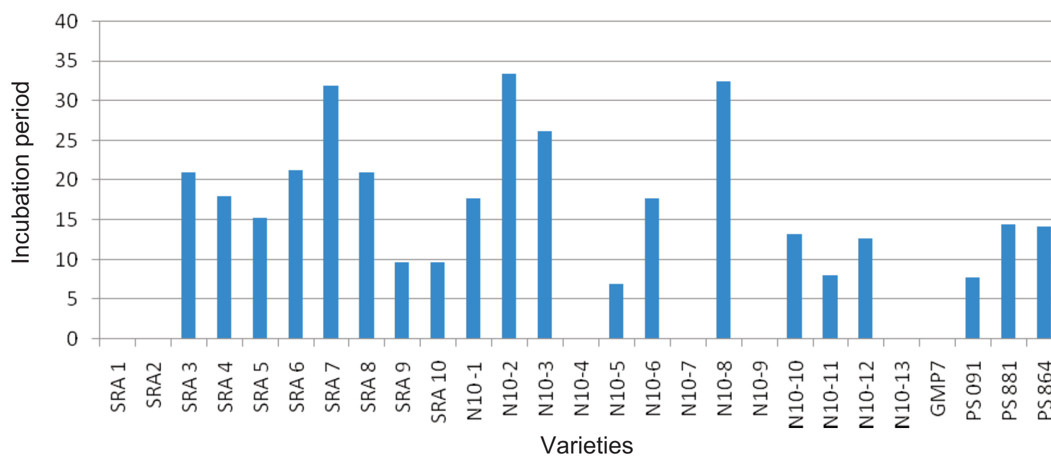

Figure 1. Average incubation period of streak mosaic disease on 27 varieties of sugarcane

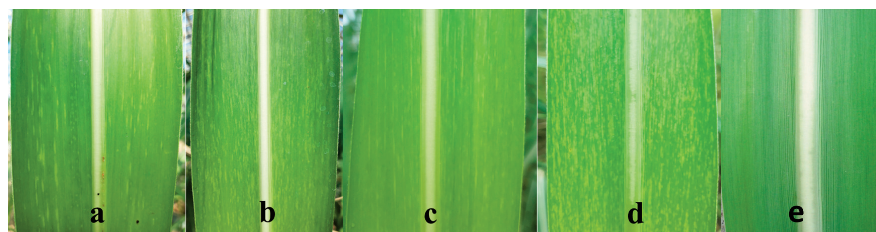

Figure 2. Phenotypes of *Sugarcane streak mosaic virus* (ScSMV) symptoms; (a) short chlorotic lines spread on leaf surface; (b) short chlorotic lines that were close together and almost covered the entire leaf surface; (c) mild chlorotic lines almost covering the entire leaf surface; and (d) slightly elongated and very clear chlorotic lines; (e) asymptomatic leaf

that streak pattern was formed short lines to rather long, greenish yellow in color. The symptoms are easily recognized on young leaves; early symptoms appear on spindle leaves, and the symptoms tend to disappear on the older leaves.

Disease Incidence and Severity

The incidence and severity of streak mosaic disease at week 4 to week 12 are presented in Figure 3. At 4 weeks after inoculation (WAI), the streak mosaic symptoms were clearly observed on some varieties, namely SRA 5, SRA 6, N 10-2, N 10-3, and N 10-5, as well as on the susceptible standard varieties (PS 864 and PS 881). Even in SRA 6, disease incidence and severity reached 87.5% and 26.8%, respectively.

In the second observation (8 WAI), the disease symptoms began to appear in most of the tested varieties, attaining 20 of 23 varieties. The incidence and severity of the disease generally increased significantly from week 4 to week 8 after inoculation. In several introduced varieties, including SRA 3, SRA 5, SRA 6, N 10-2, and N 10-3, the disease incidences were more than 40% (threshold for susceptible varieties). At the same time, the disease incidences of the susceptible standard varieties, namely PS 864 and PS 881 also reached more than 40%.

At 8 to 12 WAI, there was generally no increase in both the incidence and severity of the disease. The increase of disease incidence occurred only in N 10-12 variety, while the increment of disease severity happened on SRA 3. Until week 12, there were 6 introduced varieties with the incidence and severity of the disease remaining at 0% i.e., SRA 1, SRA 2, N 10-4, N 10-7, N 10-9, N 10-13 and this was comparable with GMP 7 (resistant standard variety). The highest streak mosaic incidence and severity were shown by SRA 6 variety at 100% and 39%, respectively. In general, disease incidence rate was in line with disease severity rate, except in some varieties such as SRA 4, SRA 5, SRA 8, SRA 9, and N 10-11.

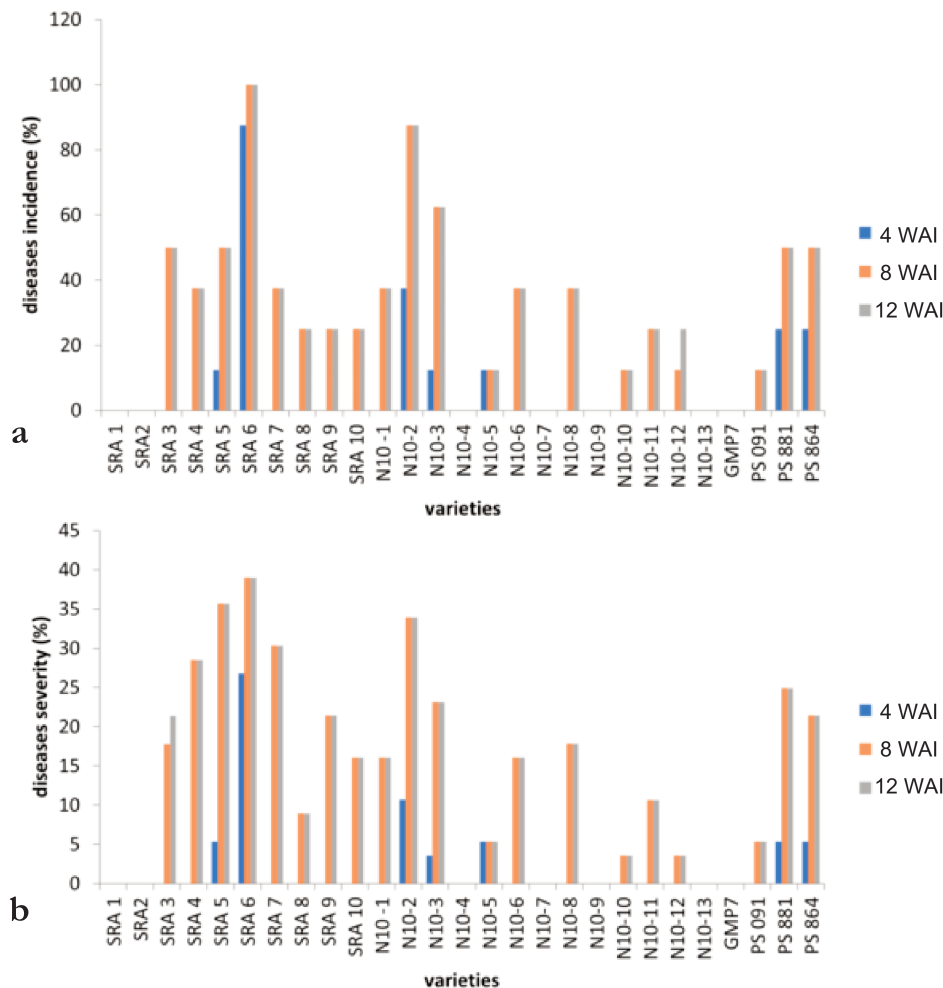

Figure 3. Streak mosaic disease incidence (a) and severity (b) of at week 4 to week 12 (WAI = weeks after inoculation)

Table 1. Disease incidence at 12 weeks after inoculation (WAI) and classification of sugarcane varieties resistance to ScSMV

No.	Varieties	**Diseases Incidence 12 WAI (%)**	**Resistance Level (scoring)**
1	SRA 1	0.0	**HR**
2	SRA 2	0.0	**HR**
3	SRA 3	50.0	**HS**
4	SRA 4	37.5	**S**
5	SRA 5	50.0	**HS**
6	SRA 6	100.0	**HS**
7	SRA 7	37.5	**S**
8	SRA 8	25.0	**S**
9	SRA 9	25.0	**S**
10	SRA 10	25.0	**S**
11	N 10-1	37.5	**S**
12	N 10-2	87.5	**HS**
13	N 10-3	62.5	**HS**
14	N 10-4	0.0	**HR**
15	N 10-5	12.5	**S**
16	N 10-6	37.5	**S**
17	N 10-7	0.0	**HR**
18	N 10-8	37.5	**S**
19	N 10-9	0.0	**HR**
20	N 10-10	12.5	**S**
21	N 10-11	25.0	**S**
22	N 10-12	25.0	**S**
23	N 10-13	0.0	**HR**
24	GMP 7	0.0	**HR**
25	PS 091	12.5	**S**
26	PS 881	50.0	**HS**
27	PS 864	50.0	**HS**

Note: 0%: Highly Resistance (HR), 0.1–5%: Resistance (R), 5.1–10%: Moderate Resistance (MR), 10.1–40%: Susceptible (S), >40%: Highly Susceptible (HS); WAI = weeks after inoculation.

Resistance Levels

Resistance levels of the tested varieties against ScSMV based on the disease incidence at 12 WAI were presented in Table 1. In general, the disease incidence rates of the standard varieties against ScSMV were still in line with their resistance level, except on the moderate standard variety PS 091 where in this trial was 12.5% and it was slightly above 10% and categorized as susceptible varieties. The difference was more likely due to environmental factors. In this study, the experiment was carried out on a greenhouse scale, while the resistance trials for releasing varieties should also be conducted on a field scale.

There were six introduced varieties classified as highly resistant varieties, namely SRA 1, SRA 2, N 10-4, N 10-7, N 10-9, and N 10-13. Meanwhile, 12 introduced varieties were susceptible, namely SRA 4, SRA 7, SRA 8, SRA 9, SRA 10, N 10-1, N 10-5, N 10-6, N 10-8, N 10-10, N 10-11, and N 10-12, and five introduced varieties were highly susceptible, namely SRA 3, SRA 5, SRA 6, N 10-2, and N 10-3. None of the introduced varieties were categorized as resistant or moderately resistant variety.

ScSMV Detection Using RT-PCR (Reverse Transcription Polymerase Chain Reaction)

RT-PCR successfully amplified a 500 bp DNA fragment from the tested samples (Figure 4). The results of RT-PCR test showed that the collection plants of PS 864 variety used as inoculum sources and the tested plants showing mosaic symptoms in this experiment were positively infected by ScSMV.

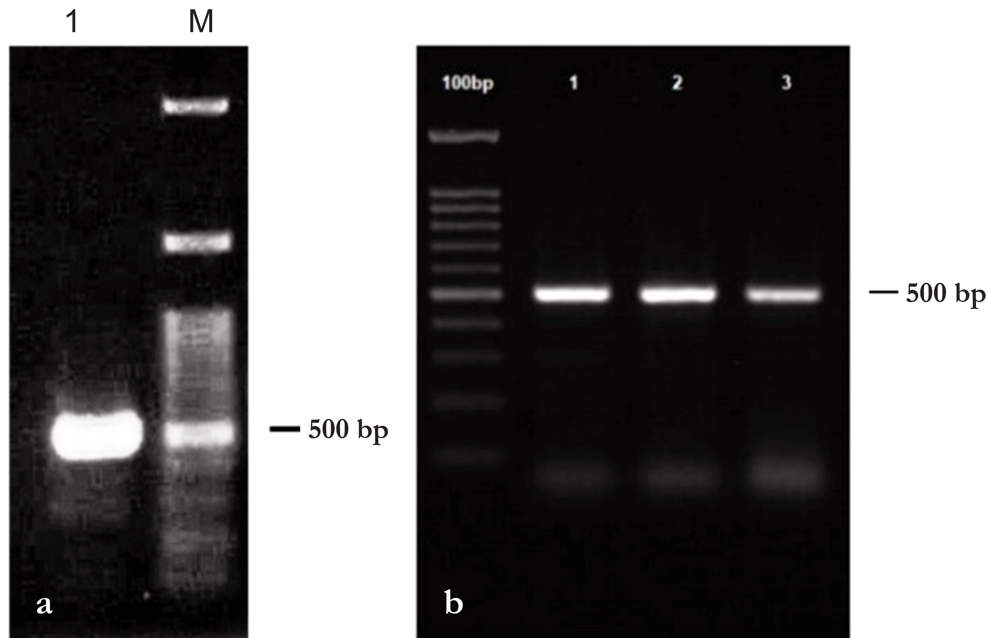

Figure 4. Results of RT-PCR electrophoresis gel; (a) PS 864 source of inoculum, (b) sugarcane samples that showed streak mosaic symptoms, lane 1 = SRA 8, lane 2= SRA 9, lane 3 = N 10-10, M = Marker

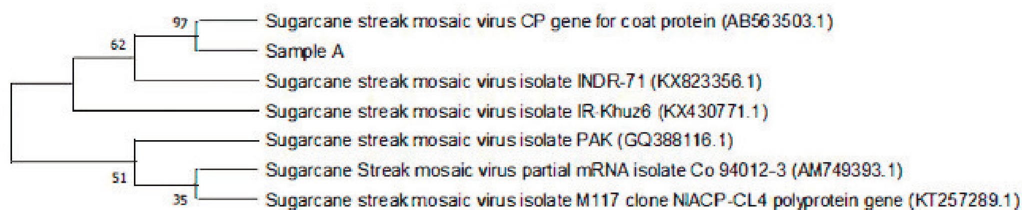

Figure 5. Phylogenetic tree constructed using Mega 10 showing relationship among some ScSMV isolates

Phylogenetic Analysis

The phylogenetic analysis showed that the inoculum source of this study (Sample A), had high sequence homology with the CP gene of ScSMV (AB563503.1), attaining 98% (Figure 5). Therefore, the virus amplified in Sample A is closely related to ScSMV from Indonesia and distinct from the isolates of other countries.

Chlorophyll Content

The results of chlorophyll content analysis showed that ScSMV infection caused a decrease in chlorophyll content in PS 091, PS 864, PS 881 and N 10-10 varieties, whereas the chlorophyll content increased in GMP 7 and SRA 9 varieties (Figure 6). In general, there was a tendency of lower chlorophyll content in infected plants compared to healthy plants. These results agree with the previous research findings. Sholeh *et al.* (2019) and Hamida & Suhara (2019) stated that mosaic infection causes the decrease of sugarcane chlorophyll contents. However, different physiological responses may occur depending on the variety as happened in GMP 7 and SRA 9.

Proline Content

The results of proline measurement revealed that healthy plants tended to have higher proline levels compared to diseased plants, except for GMP 7 (Figure 7). Results showed that ScSMV infection decreased proline levels and this was in disagreement with findings from previous experiments showing virus infection stimulated the increase of proline accumulation in leaves plants (Mohanty & Sridhar, 1982; Pazarlar *et al.*, 2013; Bassiouny *et al.*, 2015; Soni *et al.*, 2022). However, the results of this study were in line with the findings of Hosseini *et al.* (2021). These differences may be caused by genetic factors of each plant and also how plants deal with stress under different environmental status. Aldila *et al.* (2008) stated that proline accumulation is propor-

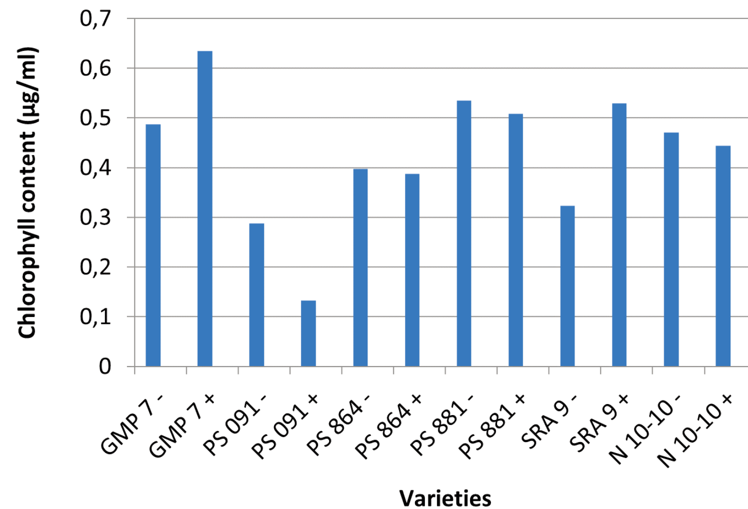

Figure 6. Chlorophyll content in asymptomatic (-) and symptomatic (+) sugarcane leaves

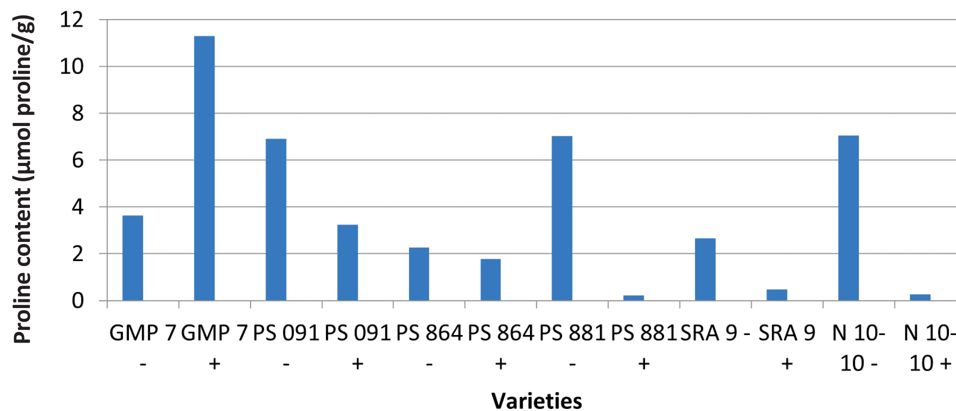

Figure 7. Proline content in asymptomatic (-) and symptomatic (+) sugarcane leaves

tional to water stress intensity, but due to plant genetic variations, a positive correlation is not always found. In addition, the different viruses may activate different resistance reactions in plants. Mandadi *et al.* (2013) found that in some experiments, proline production may not be activated by virus inoculation.

Discussion

Streak mosaic symptoms were observed in several tested varieties with mosaic pattern symptoms on leaves in the form of greenish-yellow short patches and the symptoms were more pronounced on younger leaves. This symptom is the same as previously described by Damayanti and Putra (2011) and Putra *et al.* (2014). Streak mosaic symptoms on several tested varieties varied and there were four different types of symptoms. Variations of streak mosaic symptoms were also reported by Putra and Damayanti (2012) that showed three variations of symptoms. RT-PCR assay confirmed that the tested varieties showing mosaic symptoms and the diseased plants used as inoculum sources in this experiment were infected by ScSMV

This study revealed that the incubation period of the disease for mosaic symptoms to appear after inoculation ranged between 7–34 days, and susceptible control varieties, namely PS 864 and PS 881, showed the symptoms at about 14 days after inoculation. Damayanti *et al.* (2010) also reported that the incubation period of ScSMV in PS 864 was ± 14 days.

On the first observation (4 WAI), 30% of varieties showed streak mosaic symptoms, while on the second observation (8 WAI) around 74% of varieties were infected with ScSMV. There were almost no increase of varieties infected with ScSMV on the third observation (12 WAI). Therefore, the disease incidence

and intensity on the second observation determined the level of resistance, especially varieties that have shown the symptoms. Varieties that show no mosaic symptoms until 12 WAI were categorized as highly resistant varieties. Putra (2015) recommended that for resistance trials, disease observations should be carried out until 3 months after inoculation due to disease incidences remain stable over that period, and symptom observations becomes technically difficult as sugarcane plants have grown tall.

The resistance trial revealed that most of the tested varieties both from Australia (SRA code) and Brazil (N code) were susceptible and highly susceptible to ScSMV. The highly resistant varieties to ScSMV were SRA 1, SRA 2, N 10-4, N 10-7, N 10-9, and N 10-13. These highly resistant varieties can potentially be used to replace susceptible commercial varieties after passing adaptation and preliminary yield tests in the field. In addition, they can be utilized as a parent in sugarcane breeding programs to obtain high yielding varieties resistant against ScSMV.

The resistance response of each sugarcane variety to ScSMV is different. The mechanism of plant resistance to viruses is determined by the presence of plant inhibition in viral replication and systemic translocation in plant tissues (Matthews, 1991; Valkonen, 2002). In susceptible varieties, there are no plant inhibition mechanism against viral replication and systemic translocation from the point of inoculation to other parts of the plant. Conversely, the plant inhibition ability against viral replication and speed of virus movement occurred in resistant varieties. Putra *et al.* (2003) found that in moderately resistant sugarcane varieties that were mechanically infected with SCMV, systemic distribution of the virus in plant tissues is inhibited causing mosaic symptoms to appear later compared to susceptible varieties. Furthermore, Bedoya *et al.* (2011) explained that the inhibition of SCMV translocation is a critical factor in the resistant varieties.

Virus infection causes physiological alterations in infected plants, including the content of chlorophyll and proline on the leaves (Chatterjee & Ghosh, 2008; Pazarlar *et al.*, 2013; Zhao *et al.*, 2016). ScSMV infection causes chlorosis symptoms on the leaves and Agrios (2005) stated that the symptoms reflect the damage or inhibition of chlorophyll formation. This study found that the chlorophyll content tends to decrease. The reduction of chlorophyll contents indicated that virus particles in leaf mesophyll tissue has damage the chloroplasts causing a mosaic pattern. A viral infection is suspected to cause structural changes in the chloroplast, resulting in disruptions in chlorophyll synthesis (Pazarlar *et al.*, 2013; Zhao *et al.*, 2016).

Proline accumulation is a common metabolic response of plants to both abiotic and biotic stress. Previous experiments found that virus-infected plants increased proline content in plant tissues (Mohanty & Sridhar, 1982; Pazarlar *et al.,* 2013; Bassiouny *et al.*, 2015; Soni *et al.*, 2022). Chen and Dickman (2005) described that when plants are infected by microbial pathogen, they produce reactive oxygen species (ROS) that induced programmed cell death in the plant cells at the infection site to effectively wall off the pathogen and terminate the disease process. Proline may act as a powerful scavenger of ROS and its property might prevent the induction of programmed cell death by ROS. Conversely, the result of this study found that the proline content of ScSMV-infected sugarcane decreased compared to the healthy plants. It is suspected that ScSMV infection in sugarcane is negatively correlated with proline accumulation. The different results might be caused by the genetic differences of both plants and viruses as well as different water stress conditions. However, it needs to be confirmed with further experiments. Mohanty and Sridhar (1982) explained that the increase of proline in virus-infected plants is also affected by abiotic stress and is positively correlated with plant susceptibility to viruses. Proline production is associated with plant genotype variations causing physiological response among plants will differ (Aldila *et al.*, 2008) and proline synthesis under stress conditions is a gene regulated process involving the activation of genes of its biosynthesis (Sumitra & Reddy, 2004). Mandidi *et al.* (2013) found that in some experiments, proline production may not be activated by virus inoculation and proline might be degraded to facilitate virus resistance.

Varieties resistance responses to viral infection is a complex mechanism because it involves plant variety, strain virus and the environment (Matthews, 1991; Valkonen, 2002). Results of this experiment were an early indication of resistance response of several sugarcane varieties to ScSMV infection. The highly

resistant varieties obtained from this glasshouse experiment are not recommended for direct planting in the field. For this reason, it is suggested to carry out further field scale studies to determine resistance under complex field environments.

CONCLUSION

The introduced varieties tested in this study showed different resistance responses and the majority of them were susceptible to ScSMV. There were six highly resistant varieties against ScSMV, namely SRA 1, SRA 2, N 10-4, N 10-7, N 10-9, and N 10-13. However, further investigation is required before they are applied for controlling ScSMV.

ACKNOWLEDGEMENT

The author thanks the Indonesian Sugar Research Institute (ISRI) technical staff, the Australian Center for International Agricultural Research (ACIAR) for funding and support, also the Center for Estate Crop Seed and Protection of Surabaya in Jombang for laboratory facilities.

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