Screening sugarcane accessions for resistance against Sugarcane Streak Mosaic Virus (SCSMV)

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Abstract. Sugarcane Streak Mosaic is a new sugarcane disease and has widespread sugarcane fields in Java, some parts of Sumatra, and Sulawesi. A severe infection could decrease sugar content and production significantly. This study aimed to screen the resistance level of eighteen potential accessions derived from cross-breeding sugarcane varieties to the disease. The research was conducted at a randomized block design (RBD) with three replications using artificial inoculation. Each accession contained 10 plants. The resistant category based on disease incidence and severity was observed 2 months after inoculation. Results of the screening indicated that four accessions were categorized as resistant, i.e: PS 04 303, PS 04 199, PS 06 395, and PS 06 188); 1 moderately resistant (PS 05 370); 7 accessions were susceptible and four accessions were very susceptible to SCSMV. The screening showed that there were resistant accessions that could be developed as new potential varieties.

Keywords: Saccharum officinarum hybrid, sugarcane disease, resistant

1. Introduction
In 2005 a new mosaic known as a streak mosaic disease on sugarcane plantations in Java was reported by Kristini et al. [1]. The disease was caused by Sugarcane Streak Mosaic Virus (SCSMV), a new virus, a member of a new genus in the family Potyviridae [2]. The virus was first discovered infected sugarcane in Pakistan by Hall et al. [3] and then spread to India, Bangladesh, Sri Lanka, Thailand, and Vietnam [4]. The symptoms of mosaic streak are a mosaic pattern between short light green or yellowish streak parallel to the vein bundles and normal green on the leaf blade [1]. This disease is a systemic disease since the virus will spread to all plant tissues once the virus infects the sugarcane plant. In India, almost 100% of mosaic disease is reported to have decreased in sugarcane productivity [5]. Clone culture of virus-resistant sugarcane has successfully controlled mosaic disease after identifying diseased plants [6]. The success of viral pathogens infecting plants is influenced by plant age. Mature plants with less active metabolism are more resistant to pathogenic infections than young plants [7], because the metabolic processes of young plants are very active, thus supporting multiplication and the process of viral infection.

Field surveys conducted in 2007 showed that the disease was widespread across the java island and infected almost all commercial varieties, particularly PS 864 with severity ranges of 0.28 – 62.18% [8]. The disease causes a significant reduction in cane and sugar yields. Therefore, a strategy for controlling the virus needs to be developed. The most reliable control method was using resistant variety. Therefore, this study aimed to screen some potential accessions for resistance against Sugarcane Streak Mosaic Virus (SCSMV).
2. Materials and methods
The resistance screening to streak mosaic was evaluated in 18 sugarcane accessions, i.e: PS 04 162, PS 04 117, PS 04 125, PS 04 259, PS 04 129, PS 04 194, PS 05 124, PS 05 258, PS 06 391, PS 04 303, PS 06 199, PS 06 103, PS 06 204, PS 05 370, PS 06 395, PS 06 188, BL (considered as moderate resistant variety), PS 864 (susceptible variety check).

The experiment was conducted in a randomized block design with three replicates. Each accession was planted in 10 polybags contained @ 25 kg sterilized soil. Two-eye cuttings were planted in each polybag. The viability of cane cuttings was observed by counting the number of stools at one month after planting. Virus inoculum was obtained from naturally SCSMV symptomatic young leaves. The leaves were then inoculated repeatedly until the new healthy inoculated leaves showed typical streak mosaic disease symptoms. The leaves were then crushed in 0.01 M phosphate buffer, pH 7.2 at 4°C, at a 1:5 (mg: mL) ratio. The abrasive pad rubbing method was used for SCSMV inoculation at six weeks after planting on the fully opened youngest leaves of each tested accession [9]. Disease incidence (DI) was recorded weekly started from 1–12 weeks after inoculation. The percentage of DI was calculated based on the proportion of the number of diseased tillers and the total number of tillers [10]. Classification of resistance level was adapted from the resistance grading scale of smut since both of them are systemic diseases. The levels were: resistant with disease incidence 0-5 %, moderately resistant: 5.1–15 %, moderately susceptible 15.1–30 %, and susceptible >30 % [11].

Besides DI, the appearance of streak mosaic symptoms on five young leaves was visually examined to evaluate the disease severity (DS). Percentage of DS was evaluated based on symptom grade scale based on Silva et al. [12], as follows: no symptom (0); mild mosaic in one or more leaves (1); intense mosaic in two or more leaves (2); generalized intense mosaic, along with a reduction in plant growth (3). However, DS data were not used to categorize resistant levels. DS evaluation was used to support DI. The presence of SCSMV was confirmed by polymerase chain reaction (PCR) 12 weeks after inoculation using a specific primer for SCSMV (AP3/547F) [13]. The number of tillers was also recorded at the age of five months old from each sugarcane accession.

3. Results and discussion
3.1. Disease incidence
The disease incidence of SCSMV on the tested accessions was ranged between 0-100 % during 12 times of observation. Table 1 shows the disease incidence development every two weeks to simplify the table and it did not affect the category of their resistance level.

Table 1. Disease incidence of 18 tested sugarcane accessions and their resistance level against SCSMV.

| Accession | 2   | 4   | 6   | 8   | 10  | 12  | Resistance level |
|-----------|-----|-----|-----|-----|-----|-----|------------------|
| PS 04 162 | 0.0 | 0.0 | 6.7 | 73.3| 76.7| 76.7| Susceptible      |
| PS 04 117 | 23.3| 80.0| 80.0| 90.0| 90.0| 93.3| Susceptible      |
| PS 04 125 | 0.0 | 23.3| 66.7| 93.3| 93.3| 96.7| Susceptible      |
| PS 04 259 | 16.7| 86.7| 93.3| 96.7|100.0|100.0| Susceptible      |
| PS 04 129 | 0.0 | 10.0| 46.7| 86.7| 93.3| 96.7| Susceptible      |
| PS 04 194 | 26.7| 93.3| 93.3| 96.7|100.0|100.0| Susceptible      |
| PS 05 124 | 3.3 | 80.0| 96.7| 96.7| 96.7| 96.7| Susceptible      |
| PS 05 258 | 6.7 | 50.0| 76.7| 90.0| 90.0| 93.3| Susceptible      |
| PS 06 391 | 6.7 | 16.7| 40.0| 86.7| 86.7| 93.3| Susceptible      |
| PS 04 303 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | Susceptible      |
| PS 06 199 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | Susceptible      |
| PS 06 103 | 26.7| 80.0| 90.0| 90.0| 93.3| 93.3| Susceptible      |
| PS 06 204 | 20.0| 73.3| 86.7| 90.0| 93.3| 93.3| Susceptible      |
| PS 05 370 | 23.3| 43.3| 80.0| 83.3| 86.7| 86.7| Susceptible      |
| PS 06 395 | 0.0 | 0.0 | 3.3 | 3.3 | 3.3 | 3.3 | Resistant        |

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3.2. Disease severity

The disease severity of SCSMV was calculated based on the symptom level of the top five leaves of each accession to support the disease incidence (Table 2.).

Table 2. Disease severity of 18 tested sugarcane accessions against SCSMV.

| Accession     | Week Observation (%) |
|---------------|----------------------|
|               | 2        | 4        | 6        | 8        | 10       | 12       |
| PS 04 162     | 0.0      | 0.0      | 16.8     | 19.2     | 21.7     | 23.0     |
| PS 04 117     | 0.0      | 27.0     | 32.5     | 37.8     | 43.7     | 46.7     |
| PS 04 125     | 24.4     | 26.4     | 30.7     | 34.5     | 37.1     | 39.2     |
| PS 04 259     | 43.7     | 48.3     | 56.3     | 66.9     | 71.9     | 76.4     |
| PS 04 129     | 0.0      | 31.9     | 37.0     | 40.0     | 42.7     | 45.1     |
| PS 04 194     | 54.5     | 58.1     | 63.0     | 70.1     | 75.0     | 82.1     |
| PS 05 124     | 41.2     | 44.5     | 49.4     | 52.4     | 56.0     | 61.0     |
| PS 05 258     | 15.6     | 18.9     | 21.2     | 23.4     | 25.8     | 27.3     |
| PS 06 391     | 4.2      | 5.4      | 6.1      | 8.9      | 11.8     | 14.3     |
| PS 04 303     | 0.0      | 0.0      | 2.5      | 3.3      | 4.1      | 5.0      |
| PS 06 199     | 0.0      | 0.0      | 2.0      | 2.9      | 3.6      | 4.4      |
| PS 06 103     | 39.1     | 44.1     | 48.8     | 55.6     | 60.0     | 67.2     |
| PS 06 204     | 14.4     | 17.7     | 21.4     | 24.0     | 27.9     | 30.9     |
| PS 05 370     | 4.2      | 4.7      | 5.2      | 5.5      | 6.5      | 7.2      |
| PS 06 395     | 0.0      | 0.0      | 2.0      | 3.0      | 4.5      | 4.9      |
| PS 06 188     | 0.0      | 0.0      | 0.2      | 0.8      | 1.1      | 1.5      |
| BL (Moderate) | 0.0      | 0.0      | 0.0      | 0.0      | 0.0      | 0.0      |
| PS 864 (Susceptible) | 40.7     | 43.5     | 45.3     | 46.4     | 50.5     | 53.0     |

Figure 1. Correlation between disease incidence and disease severity.
There was a strong correlation \( r=0.9078 \) between disease incidence and disease severity, meaning that the higher the disease incidence, the higher the disease severity (Figure 1). Only PS05370 has low disease severity (7.2%) but very high disease incidence (86.7%). SCSMV is a systemic disease. Thus, the incidence of the disease indicates that once sugarcane cane stalk is infected, the entire stem contains SCSMV. The number of viruses in the tissue could be expressed by the severity of the symptoms. The more intense the mosaic symptoms, the chlorophyll loss will also be higher. These conditions could affect the process of photosynthesis in the leaves. The more chlorophyll is lost, the lower the rate of photosynthesis to produce energy or carbohydrate for the plant to grow and produce tiller or sugar. As reported by [14, 15], chlorophyll is the main factor in the photosynthesis process of the sugarcane crop.

3.3. PCR confirmation

The presence of SCSMV in plant was confirmed by PCR analysis, except for the resistant accessions (Table 3, Figure 2). DNA bands according to the amplification target (~547 bp) were successfully obtained from 13 samples derived from susceptible tested accessions, including variety PS 864 as susceptible check, i.e: PS 04 162, PS 04 117, PS 04 125, PS 04 259, PS 04 129, PS 04 194, PS 05 124, PS 05 258, PS 06 391, PS 06 103, PS 06 204, and PS 06 370. Target DNA bands were not amplified from the other five samples derived from resistant accessions (PS 04 303, PS 06 199, PS 06 395, PS 06 188) including BL considered as moderate resistant. This PCR detection is used to confirm whether it is true that the target virus is not found in plants. So, if there was no amplified target DNA band, it is assumed that there is no viral infection. No viral infection occured possibly because of the plant had resistant response. Thus, it can be concluded that SCSMV could not be detected on resistant accessions showing that the number of viruses was very low or could not grow.

Table 3. PCR result on 18 tested sugarcane accessions using pair of 547F/AP3 primer.

| No. | Accession | PCR   | No. | Accession          | PCR   |
|-----|-----------|-------|-----|-------------------|-------|
| 1   | PS 04 162 | (+)   | 10  | PS 04 303         | (-)   |
| 2   | PS 04 117 | (+)   | 11  | PS 06 199         | (-)   |
| 3   | PS 04 125 | (+)   | 12  | PS 06 103         | (+)   |
| 4   | PS 04 259 | (+)   | 13  | PS 06 204         | (+)   |
| 5   | PS 04 129 | (+)   | 14  | PS 06 370         | (+)   |
| 6   | PS 04 194 | (+)   | 15  | PS 06 395         | (-)   |
| 7   | PS 05 124 | (+)   | 16  | PS 06 188         | (-)   |
| 8   | PS 05 258 | (+)   | 17  | BL (Moderately resistant) | (-) |
| 9   | PS 06 391 | (+)   | 18  | PS 864 (Susceptible) | (+) |

Note : (+) = there is SCSMV detected in the sample, (-) = there is no SCSMV detected in the sample

Figure 2. Visualization of DNA amplified SCSMV using specific primers on TBE agarose gel. Target DNA amplicons ~500 bp. M, 1 kb DNA marker; sample no. 1 to 18 is sugarcane accession no 1 to 18; K+ positive control; K- is negative control(water).
3.4. Number of tiller

The highest number of tillers was found in PS 864 accession (18.7), while the lowest number was found in PS 05 124 (5.4) (Figure 3). Although there was a tendency for a higher disease incidence or disease severity, the fewer the number of tillers, but the correlations were not significant (Figure 4). It could be indicated that the ability of the sugarcane plant to produce tillers was not affected by disease incidence or severity. It seemed that the ability to produce tillers was their own potential genetic. Kandel et al [16] reported that tillering in sugarcane was controlled by a tb1 gene. PS06 188 was categorized as a resistant accession and it has the highest number of tillers among the tested accessions (14.4), followed by PS 04 199 (11.1) and PS 06 395 (10.7). Although accession PS 04 303 was resistant, its tiller production was low (7.6).

![Figure 3](image1.png)

**Figure 3.** The number of tillers produced by 18 tested sugarcane accessions.

![Figure 4](image2.png)

**Figure 4.** Correlation between the number of tillers and disease incidence amongst sugarcane accession tested.
Figure 5. Correlation between the number of tillers and disease severity.

4. Conclusion

Based on disease incidence, four accessions were categorized as resistant to sugarcane streak mosaic, i.e.: PS06 188, PS 04 199, PS 06 395, and PS 04 303. There was a strong correlation between disease incidence and disease severity. However, there was no correlation between disease incidence or severity and the number of tillers. Hence, the first three resistant accessions that produce a high number of tillers are good potential accessions.

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