Rapid methods for screening sugarcane clones resistance against smut disease (Sporisorium scitamineum Syd.)

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Abstract. The Indonesian government has a program to increase sugarcane productivity for domestic and export purposes. One constraint to sugarcane production is smut disease caused by Sporisorium scitamineum Syd. New resistant clones should be released since this disease already spreads to sugarcane varieties that are widely grown by farmers. Sugarcane clones are actually available, unfortunately the standard procedures for screening of clones resistance requires up to 5 mo. This experiment was conducted to develop a rapid screening method for smut resistance. The method was based on injecting 2 mL of S. scitamineum spores (10^6 spores/mL) on 4-mo-old plants. Injection was carried out on stem, 15 cm from the ground to avoid contamination; a maximum humidity of 72 hr in the inoculation area was maintained by sterile water droplets. Incidence of dried leaves and occurrence of smut spores at the leaves was observed as indication of plant responses to smut infection. With this method, plant responses can be obtained in less than 3 mo. This rapid screening method can be used as an alternative to shorten the testing period of sugarcane resistance to sugarcane smut.

1. Introduction

Sugarcane (Saccharum officinarum L.) is one of the high economic value estate crops in Indonesia for sugar production. In addition to the sugar industry, sugarcane can also be used as raw materials for other industries such as food, beverages, pharmaceuticals, chemicals, fertilizers and animal feed. According to Indonesian Directorate General of Estate Crops (2017), sugarcane in Indonesia are planted on a large scale across 10 provinces with a total land area of 453 456 ha [1]. The government increased its target from consumption sufficiency to become national sugar self-sufficiency and potential for export in 2024 [2]. Plant diseases is often reported to become a constraint for sugarcane production.

Two most important sugarcane diseases in decades are pokkah boeng (Fusarium sacchari) [3] and mosaic (Sugarcane streak mosaic virus) [4]. However, smut disease in sugarcane has recently caused a significant yield loss, i.e. up to 38% [5]. This disease, caused by the fungus Sporisorium scitamineum Syd. is widely spread in var. Bululawang (BL) and var. PS864 that commonly grown by farmers and sugar factories. Several strategies have been done to control this disease, such as the use of synthetic chemical fungicide and hot water treatment. However, the susceptible propagative materials could not inhibit this smut disease epidemic due to its ability to spread rapidly by wind.
Screening method during development of resistant varieties takes a very long time, i.e. 4 to 8 months [6-9]. This is due to the inoculation method and symptom latent period. Therefore, we developed a rapid method for screening sugarcane clones, so that the results can be obtained in less than 3 months.

2. Methods

2.1. Preparation and acclimatization of sugarcane

Screening test was carried out in Cikabayan Greenhouse, Faculty of Agriculture, IPB University. A total of 4 clones of sugarcane (C1, C2, C3, C4) were obtained from PTPN X, Pati (a national sugarcane company in Central Java). As a comparison, 2 varieties commonly grown by farmers, i.e. BL and PS 864 were included. Planting media consisted of sterile soil and compost with a ratio of 4 : 1 for each pots with 5 kg capacity. Plants maintenance was carried out by watering the plants in the morning and in the evening, weeding, and fertilizing. Seedlings were acclimatized for 30 days to facilitate optimum plant growth after planting. In addition, the acclimatization process was carried out to grow 6 leaves from sugarcane plants as an indication that the plants were ready to be inoculated. The experiment was arranged in completely randomized design with 3 replications and 11 plants for each replication.

2.2. Preparation of $S. scitamineum$ Syd. isolates

Inoculum of $S. scitamineum$ was obtained by collecting teliospores from infected plants in the field. Infected sugarcane plants were collected from PTPN X and the extraction of teliospores was carried out on the same day. Teliospores extraction was conducted by scraping sugarcane leaves covered with a mass of attached teliospores. Teliospores mass was filtered to eliminate the plant debris or other materials, then stored in refrigerator at 4°C until used [7]. Preservation of teliospores at lower temperature is not recommended because the rust teliosporic cell walls are susceptible to low temperature stresses.

The isolates that will be used for inoculation were prepared with a concentration of 2 g/L of sterile water. The amount of teliospores solution prepared is adjusted to the number of plants to be tested. In this experiment, as many as 400 mL solution (0.8 g of teliospores + 400 mL sterile water) was used for 200 plants. As many as 40 drops of Tween-20 were added to the solution for homogenization. To speed up the homogenization process, the spore solution was shaken with an incubator at a speed of 120 rpm within 12 hr.

2.3. Inoculation of $S. scitamineum$ Syd. to sugarcane

Pathogen was inoculated on stems about 15 cm from the soil surface to minimize the potential of contamination from soil microbes. The inoculation area was firstly sterilized by spraying 0.5% sodium hypochlorite (NaOCl) solution then rinsed with sterile water. A total of 2 mL of $S. scitamineum$ with a density of $5 \times 10^6$ spores/mL was injected to the plant and injection point was then covered with sterile tissue paper. The sterile tissue paper was moistened by sterile water in 0, 24, and 48 hr after inoculation to maintain a maximum humidity for spores germination.

2.4. Variables of observation

2.4.1. Proliferation. Three plants from each clones were cut and split at the inoculation point. Observation were made to find out the number of spores in the inoculation area. A significant increase in spore numbers indicates that the clones are susceptible so that the pathogen can sporulate rapidly.

2.4.2. Incubation period. The incubation period was observed from inoculation day until the first symptoms appear on the plant. The faster the symptoms appear, the more susceptible the clones will
be. Symptoms were observed in new leaves that appeared from the buds inoculated in the form of dried leaves and occurrence of smut spores at the leaves.

### 2.4.3. Disease incidence

Incidence of disease was assessed by measuring the proportion of infected plants (n) to inoculated plants (N). The resistance of sugarcane to smut disease was classified according to Rao et al. [10] with modification, i.e. addition of moderately resistant category (Table 1).

| Response           | Disease incidence (%) |
|--------------------|-----------------------|
| Resistant          | 0 < x ≤ 30            |
| Moderately resistant| 30 < x ≤ 50           |
| Susceptible        | > 50                  |

### 3. Results and discussion

Proliferation of uredospores was observed in 3 d after inoculation (dai). Significant increase in number of spores was demonstrated on BL and 864, while the increase in the number of spores in 4 tested clones was lower (Table 2). This data confirmed that many symptoms in BL and PS864 in the field is due to the proliferation rate of *S. scitamineum*.

| Clones / varieties | Number of spores (spores/mL) |
|--------------------|------------------------------|
| Bululawang         | 3.0 x 10^8                   |
| PS864              | 4.5 x 10^7                   |
| C1                 | 8.0 x 10^6                   |
| C2                 | 1.3 x 10^7                   |
| C3                 | 2.9 x 10^6                   |
| C4                 | 5.5 x 10^6                   |

Teliospores of *S. scitamineum* has rounded-shape, yellow to brown colour, with thin cell walls. After inoculation, these pathogens invaded the buds. Symptoms of the disease can be observed in new, dried leaves and the occurrence of black spore-mass at the base of the leaves (Fig. 1).

Based on the incubation period, the symptoms of BL and PS864 varieties appeared on day 4, faster than the incubation period on the 4 tested clones of 7 d. It was reported previously that the first symptom can be obtained later than 4 mo. This result is in line with previous research which reported that injection is the best method to induce the smut disease [6]. Based on this data, the 4 tested clones may have a mechanism in slowing pathogenesis of *S. scitamineum*, probably due to plants’ chemical compounds. The kind of compounds that can inhibit pathogen infections are chitinase, glucanase, pectinase, and phenolic compounds. The main phenolic compounds produced by sugarcane are gallic acid, chlorogenic acid, and ferulic acid which are correlated to antioxidant activities [11].

Three wk after inoculation, we can evaluate disease progression by measuring the disease incidence. This assessment was carried out every 3 d and was ended when the infected plants no longer shows disease progression. The result showed that, clones C1 and C4 showed disease progress higher than BL and PS864. Meanwhile, clones C2 and C6 had a low disease incidence until the end of the observation period (38 dai) (Fig. 2).
In addition, infection rates in each varieties/clones were also calculated to observe the speed of disease progression in a population. To classify the resistance rate of the tested varieties/clones, the data used were the latest disease incidence and infection rate (Table 3).

Based on plant response, C1 and C3 clones are categorized moderately resistant (Table 3). Thus, these 2 clones must be further studied before they are released. If the clones have other good characters especially in agronomic traits and suitable to be planted in many regions, C1 and C3 clones can become alternative varieties to substitute var. BL that have been grown by farmers and sugar factories up to now.
It was shown through this research that resistance evaluation of sugarcane clones can be obtained in only 3 mo. This is an important contribution for moto re rapid development of resistant varieties to cope with smut disease.

**Table 3.** The latest disease incidence, infection rate, and classification of sugarcane varieties/clones resistance to smut disease.

| Varieties / clones | Latest disease incidence (%) | Infection rate | Resistance response |
|--------------------|-------------------------------|----------------|---------------------|
| BL                 | 73.6                          | 0.107          | Susceptible         |
| PS864              | 80.2                          | 0.507          | Susceptible         |
| C1                 | 48.5                          | 0.076          | Moderately resistant|
| C2                 | 94.8                          | 0.201          | Susceptible         |
| C3                 | 38.8                          | 0.132          | Moderately resistant|
| C4                 | 75.0                          | 0.235          | Susceptible         |

4. Conclusion

The recommendation for inoculation method is as follows, inoculation of viable teliospores as many as 2 mL of spores suspension (with Tween-20 and sterile water); inoculation to the stem of 6-leaves sugarcane clones around 15 cm from the root; maintenance of humidity around inoculation point. Proliferation rate of the spores must be measured on 3rd day after inoculation to make sure that the spores could invade and colonize the plant. Screening for smut resistance can be done in 3 mo following this method.

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