Resistance evaluation of sugarcane mutants to *Sporisorium scitamineum*, the causal agent of sugarcane smut disease

N Hidayah, K S Wijayanti, M Murianingrum, T Yulianti and B Heliyanto

Indonesian Sweetener and Fiber Crops Research Institute, Malang, East Java Indonesia 65152

E-mail: noorhidaqoimun@gmail.com

Abstract. Sugarcane is one of the important crops in the world. It can produce a broad range of valuable products in the area of food, health, agriculture, and bioplastic. However, sugarcane is vulnerable to *Sporisorium scitamineum* infection. The use of resistant varieties is believed to be a proper control method for the disease. The induced mutation is one of breeding program methods that can be considered to acquire resistant varieties of sugarcane to smut disease. In 2018, we did mutation for some sugarcane varieties then in 2019 the mutants were screened for their resistance to smut disease. This research aimed to identify the resistance character of sugarcane mutants to smut disease. The inoculation method was conducted by dipping the bud into *S. scitamineum* teliospore suspension for 10 minutes at 30 °C. The buds were then planted and maintained in the polybags. The results showed that out of 41 evaluated mutants, 11 mutants were highly resistant, 9 mutants were resistant, 8 mutants were susceptible and 13 mutants were highly susceptible to smut infection. It seems that we could obtain resistant sugarcane varieties through induced mutation, however the resistance of the cane is still being evaluated during the ratoon stage.

1. Introduction

Sugarcane (*Saccharum* spp. hybrids) is one of the important crops in the world as it can produce valuable products in different economic sectors, such as food, health, agriculture (fertilizer, fodder, compost), bioplastic, etc. Sugarcane is mainly grown for sugar production. However its by-products (bagasse, molasses and press mud) can be further processed into greater economic values such as bioenergy, bioethanol and bioelectricity [1].

Sugarcane is vulnerable from pathogen infection, such as a basidiomycete fungus *Sporisorium scitamineum*, the causal agent of sugarcane smut disease. The disease can be easily recognized by the formation of a whip-like structure at the top of the sugarcane plant. This structure consists of fungal sori which are covered by a thin layer of the host tissue [2]. Once the layer is ruptured, the spores can spread to other plants and result in a new disease if the environmental condition is favorable for the disease development. The smut disease is one of important diseases on sugarcane as it can cause significant losses on susceptible varieties. It was reported that in Australia, the susceptible varieties could lose their yield up to 60% [3]. Ratoon cane is more vulnerable to smut infection rather than plant cane. The yield loss on plant cane in China could reach up to 9%, while on ratoon cane it could up to 11% [4]. Whilst in Indonesia, sugarcane smut disease has been widely spread on sugarcane plantations across the country especially in the eastern parts of Indonesia which have favorable conditions for disease development, dry and hot conditions. Therefore, the use of resistant varieties is believed to be a promising control method for this disease.
Many efforts of breeding programs have been carried out to achieve resistant varieties of sugarcane to smut disease, such as through hybridization. However, it seems that a breeding program using hybridization takes a long time to obtain that resistant cultivars. Another breeding program such as induced mutation using the physical method by gamma irradiation has been applied for improving sugarcane traits involving resistance to *S. scitamineum* infection. We did apply gamma irradiation for sugarcane in 2018 and had obtained some promising clones with good agronomic traits, such as producing lots of tillers with a minimum secondary stalk of nine. In 2019, those sugarcane mutants were screened for their resistance to *S. scitamineum* infection. Hence this study aimed to identify the resistant character of sugarcane mutants to smut disease caused by *S. scitamineum*.

2. Materials and methods
This research was carried out from April to December 2019 in Karangploso research station of Indonesian Sweetener and Fiber Crops Research Institute, Malang, Indonesia. The Karangploso research station is situated at 515 m above sea level.

2.1. Spore collection and germination assessment
The spores of *S. scitamineum* were collected from infected sugarcane plants in Karangploso research station by cutting the whips and putting them in plastic bags. The spores were harvested by scraping the whips using a plastic knife then filtered using a sieve (200 mesh) to remove plant debris then keeping them at 4 °C until used. Before used for a source of inoculum, the spores were assessed for its viability by measuring the germination percentage [5]. Initially, the spore suspension was made by adding 0.1 g of smut spores into 100 mL of sterile water then added with two drops of Tween-20. The suspension was then mixed thoroughly using a magnetic stirrer. The concentration of spores in the suspension was then determined using a haemocytometer and adjusted to 1.5x10⁶ spores per mL. A 200 µL of suspension was then spread onto Water Agar (WA) medium and maintained at 30 °C for 6 hours. After six hours of incubation, the plates were taken from the incubator then stained using lacto cotton blue. The germination percentage of smut spores was determined under a compound microscope (ZEISS West Germany) at 100x magnification. For this experiment, the spores having more than 90% germination were used for inoculation. A spore was considered to germinate if the germ tube length was at least half of the spore diameter [6].

2.2. Preparation of sugarcane bud setts and inoculation with *S. scitamineum* spore suspension
The source of sugarcane bud setts was 41 clones of sugarcane mutants, PS881 and RAD21 non-mutant as control of untreated clone (table 1). Clone numbers 1 to 29 are the mutant of PS881, while 31 to 42 are RAD21 mutant. The setts for each clone were prepared by cutting the cane stalk into 6 cm containing one bud per set. The inoculum was prepared by adding 1 g of spores into 1 L of water with ten drops of Tween-20. The concentration of spore for inoculation was determined using a haemocytometer and adjusted to 5x10⁶ spores per mL. Inoculation method used in this study was immersing the sugarcane bud setts in the spore suspension for 10 min at 30 °C. After inoculation, the bud setts were planted in the polybag, one sett per polybag, containing soil amended with cattle manure.

2.3. Disease assessment
A total of 12 plants was planted for each clone. The germination percentage of sugarcane setts was observed each week from one to six weeks after planting (wap). The number of plants showing smut disease, which was recognized by the emerging of smut whip at the top of the plant, was also recorded each week after the first symptom was appeared. The incidence of smut disease was then calculated according to this formula [5]:

\[
\text{Disease incidence} = \left(\frac{\text{diseased plants}}{\text{total number plants}}\right) \times 100
\]

Regarding the percentage of disease incidence, the resistance character of each sugarcane mutant was then rating and classified according to [7] (table 2).
Table 1. Forty sugarcane mutants screened for their resistance to smut infection.

| No. | Clone code | No. | Clone code |
|-----|------------|-----|------------|
| 1   | J22R9-10  | 23  | J14R16-70 |
| 2   | J20R2-30  | 24  | J12R1     |
| 3   | J19R11-36 | 25  | J10R9-107/162 |
| 4   | J19R5-34  | 26  | J7R6-124/106 |
| 5   | J19R2-33  | 27  | J4R11-52/64 |
| 6   | J18R2-43  | 28  | J4R6-150/159 |
| 7   | J18R17-40 | 29  | J1R6-167/4 |
| 8   | J17R12-50 | 30  | K1 (PS881Non-Mutant) |
| 9   | J17R7-49  | 31  | J20R12-337/9/2-8 |
| 10  | J17R2-46  | 32  | J19R17-325/15/3-9 |
| 11  | J16R1-57  | 33  | J16R19-351/47/6-10 |
| 12  | J16R6-56  | 34  | J14R12-230/60/8-7 |
| 13  | J16R7-55  | 35  | J13R5-206/68/9-3 |
| 14  | J15R12-65 | 36  | J7R19-105/19-9 |
| 15  | J15R4-60  | 37  | J3R12-29/143/19-6 |
| 16  | J14R1-79  | 38  | J2R19-410/152/20-10 |
| 17  | J14R2-78  | 39  | J2R4-19/419/20-2 |
| 18  | J14R4     | 40  | J1R13-157/413/21-6 |
| 19  | J14R5     | 41  | J1R20-11/154/21-10 |
| 20  | J14R7-75  | 42  | J18R10-302/28/4-5 |
| 21  | J14R10-74 | 43  | K2 (RAD21Non-Mutant) |
| 22  | J14R13-72 |     |            |

Table 2. Rating and classification of sugarcane to smut infection.

| % buds infected | Smut rating grade | General reaction |
|-----------------|-------------------|-----------------|
| Plant cane      | 0-3               | 1               | Highly resistant |
| Ratoon cane     | 0-6               | 2               | Resistant        |
| 4-6             | 7-12              | 3               | Resistant        |
| 7-9             | 13-16             | 3               | Resistant        |
| 10-12           | 17-20             | 4               | Resistant        |
| 13-25           | 21-30             | 5               | Susceptible      |
| 26-35           | 31-40             | 6               | Highly susceptible |
| 36-50           | 41-60             | 7               | Highly susceptible |
| 51-75           | 61-80             | 8               | Highly susceptible |
| 76-100          | 81-100            | 9               | Highly susceptible |

3. Results and discussion
Assessment of *S. scitamineum* viability showed that the germination of *S. scitamineum* spores collected from infected sugarcane in Karangploso research station was 93.18%. It meant that the spores were eligible to be used as a source of inoculum for this experiment.

The germinated sugarcane setts were recorded every week since one wap. The result revealed that most of sugarcane setts had germinated since one week following planting (table 3). Then the germinated setts increased every week until most of them reached up to 100% germination at six wap.
Table 3. The percentage of germinated sugarcane setts.

| Clone code        | The germinated setts at week after planting (wap) |
|-------------------|---------------------------------------------------|
|                   | 1      | 2      | 3      | 4      | 5      | 6      |
| J22R9-10          | 91.67  | 91.67  | 91.67  | 91.67  | 91.67  | 91.67  |
| J20R2-30          | 75.00  | 91.67  | 100.00 | 100.00 | 100.00 | 100.00 |
| J19R11-36         | 66.67  | 91.67  | 91.67  | 91.67  | 91.67  | 91.67  |
| J19R5-34          | 91.67  | 91.67  | 91.67  | 91.67  | 91.67  | 91.67  |
| J19R2-33          | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J18R2-43          | 91.67  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J18R17-40         | 75.00  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J17R12-50         | 33.33  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J17R7-49          | 33.33  | 83.33  | 100.00 | 100.00 | 100.00 | 100.00 |
| J17R2-46          | 41.67  | 91.67  | 91.67  | 91.67  | 91.67  | 91.67  |
| J16R1-57          | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J16R6-56          | 41.67  | 83.33  | 83.33  | 83.33  | 100.00 | 100.00 |
| J16R7-55          | 66.67  | 75.00  | 75.00  | 75.00  | 83.33  | 91.67  |
| J15R12-65         | 83.33  | 91.67  | 91.67  | 91.67  | 91.67  | 91.67  |
| J15R4-60          | 50.00  | 75.00  | 75.00  | 83.33  | 91.67  | 91.67  |
| J14R1-79          | 66.67  | 91.67  | 91.67  | 91.67  | 91.67  | 91.67  |
| J14R2-78          | 66.67  | 83.33  | 91.67  | 91.67  | 91.67  | 91.67  |
| J14R4             | 83.33  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J14R5             | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J14R7-75          | 91.67  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J14R10-74         | 41.67  | 83.33  | 83.33  | 83.33  | 100.00 | 100.00 |
| J14R13-72         | 75.00  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J14R16-70         | 50.00  | 91.67  | 83.33  | 91.67  | 91.67  | 100.00 |
| J12R1             | 83.33  | 83.33  | 83.33  | 83.33  | 91.67  | 100.00 |
| J10R9-107/162     | 41.67  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J7R6-124/106      | 50.00  | 83.33  | 91.67  | 100.00 | 100.00 | 100.00 |
| J4R11-52/64       | 75.00  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J4R6-150/159      | 83.33  | 91.67  | 83.33  | 83.33  | 91.67  | 91.67  |
| J1R6-167/4        | 66.67  | 83.33  | 83.33  | 83.33  | 91.67  | 91.67  |
| K1 (PS881NonMutan)| 75.00  | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| J20R12-337/9/2-8  | 8.33   | 75.00  | 83.33  | 83.33  | 100.00 | 100.00 |
| J19R17-325/15/3-9 | 8.33   | 66.67  | 66.67  | 91.67  | 100.00 | 100.00 |
| J16R19-351/47/6-10| 33.33  | 100.00 | 91.67  | 100.00 | 100.00 | 100.00 |
| J14R12-230/60/8-7 | 41.67  | 66.67  | 75.00  | 75.00  | 91.67  | 100.00 |
| J13R5-206/68/9-3  | 25.00  | 58.33  | 83.33  | 91.67  | 100.00 | 100.00 |
| J7R19-105/19-9    | 0.00   | 58.33  | 83.33  | 83.33  | 100.00 | 100.00 |
| J3R12-29/143/19-6 | 25.00  | 83.33  | 83.33  | 83.33  | 100.00 | 100.00 |
| J2R19-410/152/20-10| 8.33   | 66.67  | 75.00  | 75.00  | 91.67  | 91.67  |
| J2R4-19/149/20-2  | 33.33  | 83.33  | 83.33  | 83.33  | 100.00 | 100.00 |
| J1R13-157/413/21-6| 8.33   | 41.67  | 50.00  | 58.33  | 91.67  | 91.67  |
| J1R20-11/154/21-10| 25.00  | 75.00  | 100.00 | 100.00 | 100.00 | 100.00 |
| J18R10-302/28/4-5 | 16.67  | 58.33  | 75.00  | 83.33  | 100.00 | 100.00 |
| K2 (RAD21NonMutan)| 41.67  | 66.67  | 83.33  | 83.33  | 91.67  | 100.00 |
In addition, after six wap, more than 50% of sugarcane clones had 100% germination (figure 1). This result indicated that induced mutation does not affect viability of the mutant.

![Range of germination](image)

**Figure 1.** Germination of sugarcane mutant after six wap.

The first symptom of smut disease appeared seven weeks after inoculation (wai). The symptom was easily recognized by the formation of a whip-like structure at the top of the sugarcane stalk. After the first symptom emerged, the number of the infected stalk was then recorded until 17 wai. At the end of observation, it was recorded that 11 mutants were highly resistant, 9 mutants were resistant, 8 mutants were susceptible and 13 mutants were highly susceptible to smut infection (table 4).

Out of 41 sugarcane mutants evaluated, 11 mutants were highly resistant, 9 mutants were resistant, 8 mutants were susceptible and 13 mutants were highly susceptible to smut infection. This result indicated there is an opportunity to achieve resistant varieties of sugarcane to smut fungus through the induced mutation method. However, the resistant clones should be evaluated at the ratoon cane (RC) stage to ensure that they still have resistance to smut. Most sugarcane clones perform resistance at plant cane (PC), but they are susceptible at ratoon. In addition, the incidence of smut is also higher at ratoon cane rather than plant cane [4]. The yield loss of sugarcane caused by smut fungus on ratoon cane could reach up to 30% of total yield loss, depending on the resistance of sugarcane clones to smut infection [4, 8].

The incidence of smut disease on sugarcane is influenced by environmental factors especially high temperature and low relative humidity [9, 10]. Further, [9] revealed that in dry soil, teliospores of *S. scitamineum* might be able to survive more than 200 days and germinate up to 70%, in contrast, in wet soil the teliospores unable to resist more than 48 hours. In addition, [10] reported that the optimum temperature for *S. scitamineum* teliospore germination is 30 °C. [11] and [12] revealed that high temperature ranging from 30-35 °C is favorable for smut disease development in the field. Furthermore, characteristics of sugarcane varieties, resistant or susceptible, also highly influence the occurrence of smut infection [13]. [11] stated that difference resistance of sugarcane clones to *S. scitamineum* might result in various incubation periods ranged from 1.5 to 8 months as well as production of sorus.

Resistance of sugarcane to smut fungus is believed to be driven by two types of mechanisms, external and internal resistance mechanisms [14, 15, 16]. The external resistance might involve the structural morphology of sugarcane buds that physically inhibits the smut fungus to penetrate sugarcane tissues for example the presence of wax at the surface of bud and trichome [17, 18, 19]. Besides the morphology of buds, secondary metabolites produced by the bud also influence the external resistance of sugarcane to smut infection [20, 21]. The internal resistance occurs within sugarcane tissue is governed by the interaction between sugarcane and *S. scitamineum* infection after the fungus breakthrough the external
resistance [22]. This is complex mechanism involving various resistance genes and secondary metabolites pathways including phenol and pathogenesis-related protein accumulation [23, 24].

Table 4. The incidence of smut disease and resistance character of 43 sugarcane clones to \textit{S. scitamineum}.

| Clone code | Smut incidence (%) | Smut rating grade [7] | Resistance character [7] |
|------------|--------------------|------------------------|--------------------------|
| J22R9-10   | 9.09               | 4                      | Resistant                |
| J20R2-30   | 41.67              | 7                      | Highly susceptible       |
| J19R11-36  | 9.09               | 4                      | Resistant                |
| J19R5-34   | 18.18              | 5                      | Susceptible              |
| J19R2-33   | 16.67              | 5                      | Susceptible              |
| J18R2-43   | 58.33              | 8                      | Highly susceptible       |
| J18R17-40  | 41.67              | 7                      | Highly susceptible       |
| J17R12-50  | 25.00              | 5                      | Susceptible              |
| J17R7-49   | 8.33               | 3                      | Resistant                |
| J17R2-46   | 27.27              | 6                      | Highly susceptible       |
| J16R1-57   | 25.00              | 5                      | Susceptible              |
| J16R6-56   | 16.67              | 5                      | Susceptible              |
| J16R7-55   | 18.18              | 5                      | Susceptible              |
| J15R12-65  | 27.27              | 6                      | Highly susceptible       |
| J15R4-60   | 20.00              | 5                      | Susceptible              |
| J14R1-79   | 27.27              | 6                      | Highly susceptible       |
| J14R2-78   | 9.09               | 4                      | Resistant                |
| J14R4      | 50.00              | 7                      | Highly susceptible       |
| J14R5      | 66.67              | 8                      | Highly susceptible       |
| J14R7-75   | 58.33              | 8                      | Highly susceptible       |
| J14R10-74  | 8.33               | 3                      | Resistant                |
| J14R13-72  | 16.67              | 5                      | Susceptible              |
| J14R16-70  | 36.36              | 7                      | Highly susceptible       |
| J12R1      | 63.64              | 8                      | Highly susceptible       |
| J10R9-107/162 | 8.33            | 3                      | Resistant                |
| J7R6-124/106 | 9.09             | 4                      | Resistant                |
| J4R11-52/64 | 50.00            | 7                      | Highly susceptible       |
| J4R6-150/159 | 10.00           | 4                      | Resistant                |
| J1R6-167/4 | 36.36              | 7                      | Highly susceptible       |
| K1 (PS881NonMutan) | 50.00          | 7                      | Highly susceptible       |
| J20R12-337/9/2-8 | 0.00       | 1                      | Highly resistant         |
| J19R17-325/15/3-9 | 0.00     | 1                      | Highly resistant         |
| J16R19-351/47/6/10 | 0.00     | 1                      | Highly resistant         |
| J14R12-230/60/8-7 | 9.09     | 4                      | Resistant                |
| J13R5-206/68/9-3 | 0.00      | 1                      | Highly resistant         |
| J7R19-105/19-9 | 0.00       | 1                      | Highly resistant         |
| J3R12-29/143/19-6 | 0.00      | 1                      | Highly resistant         |
| J2R19-410/152/20-10 | 0.00   | 1                      | Highly resistant         |
| J2R4-19/149/20-2 | 0.00       | 1                      | Highly resistant         |
| J1R13-157/413/21-6 | 0.00      | 1                      | Highly resistant         |
| J1R20-11/154/21-10 | 0.00     | 1                      | Highly resistant         |
| J18R10-302/28/4-5 | 0.00     | 1                      | Highly resistant         |
| K2 (RAD21NonMutan) | 9.09         | 4                      | Resistant                |
4. Conclusion
Based on our screening results, out of 41 sugarcane mutants evaluated, 11 mutants were highly resistant, 9 mutants were resistant, 8 mutants were susceptible and 13 mutants were highly susceptible to smut infection.

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