

## Response of Several Sugar Cane Varieties to Infection by *Fusarium sacchari*, The Cause of Pokkah Boeng Disease in Greenhouses



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### Abstract

The fungus *Fusarium sacchari* causes pokkah boeng, a sugarcane disease that frequently occurs in sugarcane plantations. The purpose of this study is to evaluate how different sugarcane plant varieties respond to *F. sacchari* infection, which causes Pokkah boeng disease in greenhouses. This study was conducted in the Plant Protection Laboratory and the Land Greenhouse of Halu Oleo University's Faculty of Agriculture in Kendari. A Completely Randomized Design (CRD) was employed in the study, with four treatments that were repeated five times. Each treatment included six plant units, totaling 120 polybags. Incubation period, disease incidence, disease severity, AUDPC, and durability category were the characteristics that were noted. The result of this research indicates that the V1 treatment (Kidang Kencana of variations) has the longest incubation period (up to 14 days), a disease incidence of 56.73%, and a disease severity of 22%. The kidang kencana variety also shows that the rate of development of pokkah boeng disease every week is slower, but the level of resistance category is susceptible.

**Keywords:** disease severity; *Fusarium sacchari*; resistance; sugarcane plant; varieties.

### 1. Introduction

The primary compound used to make sugar is sugarcane (*Saccharum officinarum* L.), a plant that is grown only in tropical regions. Domestic sugar demand continues to rise in line with population growth. This high population growth has not yet met the demand, leading the government to continue importing sugar (Adhiem, 2018). To reduce sugar imports, it is necessary to manage sugarcane cultivation to ensure optimum cultivation, including controlling the main pests or diseases that can always threaten sugar production (Taufik et al., 2020).

The Directorate General of Plantations stated that sugarcane production in Indonesia is still declining year on year, with 2017 production at 2.4 million tons and 2018 production at 2.4 million tons. According to the Directorate General of Plantations, Indonesia's sugarcane productivity in 2018 was 2.1 million tons (BPS, 2023). One factor inhibiting sugarcane production is high rainfall and the presence of plant disease pathogens (Subiyakto et al., 2016). Pokkah boeng disease is typically caused by the fungus *Fusarium moniliforme* (Asniah et al., 2023); (Veiga et al., 2019); *F. sacchari* was the cause of the sugarcane plantation of PT. Jhonlin Batu Mandiri, Bombana Regency (Asniah, 2024). This disease causes significant problems for sugarcane production in Indonesia and other countries, reducing yields by up to 90% (Vishwakarma et al., 2013). The main symptoms of pokkah boeng disease in sugarcane plants are characterized by malformation, namely twisting of the plant shoots, followed by chlorosis and necrosis of the leaves (Viswanathan et al., 2017). The host plants of Pokkah Boeng disease include rice, corn, sorghum, and grasses (Risthayeni et al., 2018). Transmission of the disease is generally by conidia through the air; humid, rainy weather really



supports the development of the disease (Semangun, 2001). Control of pokkah boeng disease has been widely reported, but farmers generally still rely on synthetic chemical control. Preventive control measures include the use of resistant varieties. Yunus (2000) tested the sugarcane variety PS 80-910 which is resistant to pokkah boeng using tissue culture.

One of the ways to control pokkah boeng disease, which has been widely implemented, is to use resistant plant varieties to prevent the disease from developing (Yulianti, 2020). The use of pest- and disease-resistant varieties is an easy, economical, and effective alternative for control, reducing production costs and avoiding environmental pollution with hazardous synthetic chemicals (Agrios, 2004). Testing of 169 sugarcane cultivars in China showed that 33 were highly resistant, while others were highly susceptible to pokkah boeng (Xu et al., 2023).

The Kidang Kencana (KK) variety is frequently used on sugarcane plantations in Indonesia. The sweet green sugarcane variety, PS-862, is resistant to natural attacks by shoot and stem borers and to mosaic and smut diseases. The Bulu Lawang variety is susceptible to stem borers, shoot borers, and smut, and is resistant to smut and mosaic (Rokhman *et al.*, 2014). Because of the importance of using sugarcane varieties that are resistant to pokkah boeng to maintain production, research is needed to investigate the response of several sugarcane varieties to pokkah boeng infection. This study aimed to evaluate the response of sugarcane varieties to infection by *F. sacchari*, the cause of pokkah boeng disease.

## 2. Materials and Methods

### 2.1. Research Location

This research was conducted in the Plant Protection Laboratory of the Phytopathology and Greenhouse Unit, Faculty of Agriculture, Halu Oleo University.

### 2.2. Materials and tools

The materials used in this study were sugarcane seeds, Kidang Kencana (KK) variety, Green Sweet Sugarcane variety, Red Sweet Sugarcane variety, Bulu Lawang (BL) variety, *F. sacchari* fungus, Potato Dextrose Agar (PDA) media, label paper, distilled water, plastic wrap, 70% ethanol, spirits, polybags, cow dung fertilizer, and soil. The tools used were scissors, petri dishes, scotch bottles, hemacytometers, spatulas, Bunsen lamps, analytical scales, cameras, machetes, and writing instruments.

### 2.3. Preparation of Seeding Media and Planting Media

The plant-growing medium consists of a 3:1 (v/v) mixture of soil and cow dung. The growing medium is steam sterilized to prevent contamination by other microbes. The mixed growing medium is then placed in 15 cm x 15 cm polybags.

### 2.4. Seed Preparation and Planting

The plant seeds are in the form of sugarcane cuttings of the KK variety, which is susceptible to pokkah boeng (interview with a farmer at the PT Johnlin sugarcane plantation), the BL variety, the Green sweet sugarcane variety, and the Red sweet sugarcane variety. Then, the sugarcane stalks are peeled, or the leaf husks are removed. The sugarcane seeds, which have had their leaves removed, are cut to a length of 5 cm, each with one bud, using a sterilized hacksaw. The seeds are soaked in hot water at 50 °C for 30 minutes (Febrina, 2018). After the seeds are soaked, they are then planted in polybags with the bud eyes facing sideways.

### 2.5. Preparation of *Fusarium sacchari* Suspension

The *F. sacchari* fungal isolate is a collection of the Plant Protection Laboratory stored in a refrigerator. After being revitalized from PDA medium, the isolate was cultured at room temperature for five to seven days. After that, a 7-day-old *F. sacchari* isolate was suspended by adding 10 mL of sterile distilled water to a petri dish. The surface of the isolate was ground using a spreading rod, and the conidial density of the suspension was then measured using a hemocytometer.

### 2.6. *Fusarium sacchari* Application

The pathogen was applied via injection inoculation to the internodes near the shoots of sugarcane plants, following the method described by Wang et al. (2016). The application time was 4 weeks after planting. The spore density of *F. sacchari* was  $10^6$  spores/mL using a hemocytometer. The suspension volume applied was 0.1 mL per plant.

## 2.7. Plant Maintenance

Watering, weeding, and insect control are all part of plant maintenance. Depending on the growing medium's humidity, watering is done twice a day, in the morning and the evening. Watering, weeding, and pest control are all parts of plant upkeep. Depending on the growing medium's humidity, watering is done twice a day, in the morning and the evening. Every time weeds appear on the plants, weeding is done. The manual method of controlling insect pests involves directly capturing insects on plants.

## 2.8. Research Design

The experimental design used was a completely randomized design consisting of four treatments: KK variety (V1), BL variety (V2), Green sweet sugarcane variety (V3), and Red sweet sugarcane variety (V4). Each treatment was repeated 5 times, yielding 20 treatment units.

## 2.9. Observation Variables

The observation variables were incubation period (days), disease incidence (%), severity of pokkah boeng disease (%), and the area under the disease progression curve (AUDPC). The incubation period was observed daily by observing the first appearance of pokkah boeng symptoms. Disease incidence was calculated using the formula:

$$DI = A/B \times 100\% \quad (1)$$

where DI = disease incidence (%), A = number of plants showing symptoms of pokkah boeng disease, B = number of plants observed.

The following formula is used to determine the disease's severity:

$$DS = \sum [(n_i \times v_i) / (N \times Z)] \times 100\% \quad (2)$$

where: DS is disease severity (%),  $n_i$  is number of plants in each scoring,  $v_i$  is disease attack scoring value for each individual plant, N is number of plants observed, Z is highest value of attack scoring.

Attack scoring is categorized into: category scale 0 (plants do not show any disease symptoms), 1 (leaves appear slightly chlorotic on young leaves), 2 (1%-25% of plants have chlorotic and twisted leaves), 3 (26%-50% of plants have twisted leaves and/or red spots developing in the chlorotic parts), 4 (26%-50% of plants have twisted leaves and reddish spots developing in the chlorotic parts of the leaves), 5 (51%-100% of plants have twisted, shortened leaves and plant death) (Asniah, 2024). The resistance categories of sugarcane varieties based on disease severity refer to Shan et al. (2021), namely  $DS \leq 0$  (very resistant, grade 1),  $DS = 0.1 - 10.0$  (resistant, grade 2),  $DS = 10.1 - 20$  (moderately resistant, grade 3),  $DS = 20.1 - 40$  (susceptible, grade 4),  $DS \geq 40$  (very susceptible, grade 5).

## 2.10. Data Analysis

Analysis of variance (ANOVA) was used to examine the collected data. The LSD test (Least Significant Difference) was repeated at the 95% confidence level if the analysis of variance results indicated a significant difference.

## 3. Results and Discussion

### 3.1. Incubation Period

Based on the analysis of variance, the average incubation period for pokkah boeng disease was not significantly affected by the sugarcane varieties tested. The average incubation periods are presented in Table 1.

Table 1. Average incubation period (days) for pokkah boeng disease in several sugarcane varieties

Treatment	Incubation Period (days)
Kidang Kencana variety (V1)	14
Bulu Lawang variety (V2)	9.97
Green Sweet Sugarcane variety (V3)	9.9
Red Sweet Sugarcane variety (V4)	9

Table 1 shows that the average incubation period for pokkah boeng disease in sugarcane plants did not differ significantly between varieties. Treatment V1 (Kidang Kencana) had the longest incubation period, averaging 14 days. Treatment V4 (Red Sweet Sugarcane Cultivar) had the shortest incubation period, averaging 9 days.

### 3.2. Disease Incidence

The results of observations on the incidence of pokkah boeng disease in sugarcane plants across several varieties had a very significant effect at 2, 3, 4, and 5 WAI. Table 2 shows the average incidence of pokkah boeng disease on sugarcane types at different observation times.

Table 2. Average incidence of pokkah boeng disease in several varieties of sugarcane plants

Treatment	Average Disease Incidence (%) during observation period (WAI)			
	2	3	4	5
Kidang Kencana variety (V1)	33.33 a	40.00 a	53.33 a	56.73 a
Bulu Lawang variety (V2)	36.67 a	50.00 b	56.67 b	60.00 b
Green Sweet Sugarcane variety (V3)	46.67 b	56.67 c	60.00 b	63.33 b
Red Sweet Sugarcane variety (V4)	76.67 c	86.67 d	100.00 c	100.00 c
LSD 0.05	6.00	5.21	5.47	5.03

Description: Numbers followed by the same letter in the same column indicate no significant difference at the 95% confidence level; WAI (weeks after pathogen inoculation).

Table 2 shows that the lowest average incidence of pokkah boeng disease was in treatment V1 at all observation times, namely 33.33%; 40%; 53.22%; 56.73%, respectively, and the highest was in treatment V4, namely 76.67%; 86.67%; 100%; 100%, respectively. Treatment V1 showed the lowest incidence of pokkah boeng disease, which was significantly different from the other treatments at observations 3, 4, and 5 WAI. At observation 2 WAI, the lowest average incidence of the disease was in treatment V1 (Kidang Kencana), namely 33.33%, which was significantly different from treatments V3 and V4, but not significantly different from treatment V2.

### 3.3. Disease Severity

Observations of the severity of pokkah boeng disease in sugarcane plants across several varieties showed a significant impact across all observation periods. The average severity of pokkah boeng disease in sugarcane varieties across various observation periods is presented in Table 3.

Table 3. Average severity of pokkah boeng disease on several varieties of sugarcane plants.

Treatment	Mean Disease Severity (%) at observation time (WAI)			
	2	3	4	5
Kidang Kencana variety (V1)	6.67 a	12.67 a	18.00 a	22.00 a
Bulu Lawang variety (V2)	7.33 a	12.67 a	18.67 a	29.33 b
Green Sweet Sugarcane variety (V3)	10.00 b	16.00 a	25.33 b	29.33 b
Red Sweet Sugarcane variety (V4)	20.00 c	40.67 b	55.33 c	60.00 c
LSD 0.05	3.03	4.51	4.35	3.52

Description: Numbers followed by the same letter in the same column indicate no significant difference at the 95% confidence level; WAI (weeks after pathogen inoculation).

Table 3 shows that treatment V1 had the lowest disease severity across all observation periods. The average disease severity for treatment V1 was 6.67%, significantly different from treatments V3 and V4, but not significantly different from treatment V2 at observations 2 and 4 weeks per week (WAI). At the 5-week observation (WAI), treatment V1 had a disease severity of 22%, which was significantly different from the other treatments. Treatment V4 showed the highest average disease severity, up to observations 5 weeks per week (WAI), at 60%.

### 3.4. Area Under the Disease Progression Curve (AUDPC)

AUDPC, or area under the Pokkah Boeng disease development curve, is the area of the disease affected by the development of the disease within one week. The AUDPC values are presented in Figure 1. Table 4 shows the types of sugarcane varieties resistant to pokkah boeng disease.

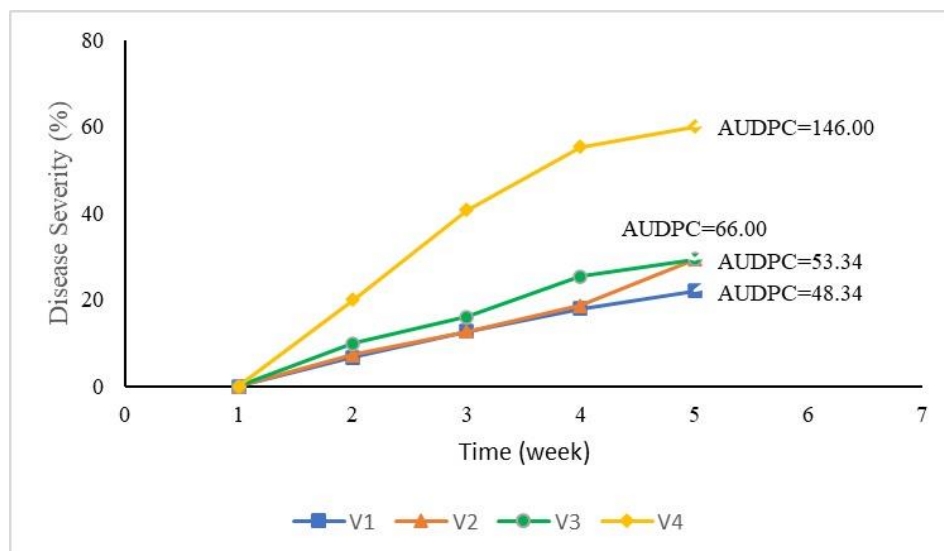


Figure 1. Pokkah boeng disease development curve per week (AUDPC) for several sugarcane varieties

Figure 1 shows the curve of the development of pokkah boeng disease, in the red sweet sugarcane cultivar (V4) the highest AUDPC value is 146 which indicates very rapid disease development so that it reaches disease severity of up to 60% in 5 WAI observations, so that the resistance of the sweet sugarcane cultivar variety is categorized as a cultivar that is very susceptible to pokkah boeng disease.

Table 4. Categories of sugarcane variety resistance to *F. sacchari* infection, which causes pokkah boeng disease in greenhouses.

Treatment	Disease Severity (%)	Grade of Resistance	Category of resistance
Kidang Kencana variety (V1)	22	4	Vulnerable
Bulu Lawang variety (V2)	29.33	4	Vulnerable
Green Sweet Sugarcane variety (V3)	29.33	4	Vulnerable
Red Sweet Sugarcane variety (V4)	60.00	5	Very Vulnerable

Description: DS ≤ 0 (very resistant, grade 1), DS = 0.1 – 10.0 (resistant, grade 2), DS = 10.1 – 20 (somewhat resistant, grade 3), DS = 20.1 – 40 (susceptible, grade 4), DS ≥ 40 (very susceptible, grade 5) (Shan et al., 2021).

Table 4 shows that all tested sugarcane varieties showed resistance to *F. sacchari* infection, which causes pokkah boeng disease, with categories ranging from susceptible to very susceptible (grades 4–5), and disease severity ranging from 22–60% across 5 WAI observations.

Pokkah boeng is one of the sugarcane diseases often found in sugarcane plantations, caused by the fungi *F. sacchari* (Ranjan et al., 2018) and *F. moniliforme* (Pratiwi et al., 2013). According to Djaenuddin and Muis (2013), the *Fusarium* pathogen can produce pectinase, metal esterase, polygalacturonase, and other destructive enzymes. These enzymes can damage cell walls, leading to growth disorders and plant diseases. Symptoms of pokkah boeng disease caused by the *F. sacchari* pathogen include chlorosis and necrosis on leaves, elongated lesions, bent or twisted leaves, reduced leaf area, and death of the upper part of the plant (shoots) (Costa et al., 2019).

The incubation period, disease incidence, and disease severity in the V1 (Kidang Kencana) treatment were lower than those in other varieties. This is believed to result from lower sugar and moisture levels in the stem tissue compared to other kinds. Morkunas & Ratajczak (2014) stated that high sugar levels stimulate the development of pathogens. The Kidang Kencana variety has a sugar content of 9.51% (Bitibalyo et al., 2021). The incubation period of a plant disease varies with interactions among host, pathogen, host developmental stage, and ambient temperature (Agrios, 2004). The red dots or lines that appear will gradually spread to all parts of the sugarcane leaf blades, and these symptoms appear around 12-30 days after inoculation (Mevianti et al., 2021). Pokkah boeng disease symptoms show on the stem as dark reddish streaks and fine lines at the nodes (Raid & Lentini, 1991).

The study's findings demonstrated that the tested sugarcane variety's resistance to pokkah boeng disease ranged from susceptible to highly susceptible, with V4 (red sweet sugarcane cultivar) in the highly susceptible group, with a disease severity of 60%. In plants susceptible to the disease, a compatible reaction occurs between the host and the pathogen, allowing the pathogen to penetrate the host plant's cell wall; conversely, in resistant plants, the reaction is incompatible (Taufik et al., 2020). The resistance of each variety can vary over time and by geographic location. The effect of each resistance gene varies from very large to very small, depending on the environment (Asniah et al., 2023). Nurhayati et al. (2010) stated that, in addition to shifts in pathogen resistance, *F. moniliforme* can also adapt to the environment, thereby breaking the resistance of previously resistant varieties. According to Syam (2008), resistance to a pathogen varies among plant varieties. The presence of different resistance genes causes this, and it is estimated that the number of resistance genes varies.

The severity of pokkah boeng disease varied among the tested varieties, with the Kidang Kencana variety showing a lower disease development rate of 48.34% in one week compared to the V4 (red sweet sugarcane) treatment, which was much higher at 146% in one week. The low AUDPC value indicates the variety's ability to limit pathogen growth in plant tissue, resulting in slower and fewer symptoms. Testing conditions in the greenhouse and in the field affected the varieties' resistance responses to pokkah boeng disease. Shan et al. (2021) conducted resistance testing of varieties in greenhouses and fields, where the highest disease severity occurred in the greenhouse at 51%, categorized as susceptible, while in field testing, 58% were categorized as resistant. Furthermore, Wang et al. (2016) conducted resistance testing in a greenhouse using the injection and spraying method, showing that the severity of pokkah boeng disease was higher in greenhouse conditions (20.02%) than in field tests on the GT37 cultivar (10.50%). Controlling pokkah boeng disease is important, as the study's findings show that the four sugarcane types span resistance categories from susceptible to highly susceptible. Control efforts can be carried out by combining several control measures, such as applying good cultivation techniques and using endophytic fungi for biological control (Asniah et al., 2025), thereby maintaining sugarcane production and meeting future sugar demand (Poorniammal et al., 2024).

#### **4. Conclusion and Suggestions**

According to the study's findings, the Kidang Kencana variety had the longest illness incubation period (14 days) observed in 5 WAI, and the lowest incidence and severity of pokkah boeng disease (56.73% and 22%, respectively). In the greenhouse, the four examined varieties were categorized as highly susceptible to pokkah boeng disease. To confirm the results from the greenhouse, more testing of more sugarcane types and field assessments are required.

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#### **CRedit Authorship Contribution Statement**

Asniah, M. Botek, M. Safitri, and F. Ganti designed and conducted the experiments and prepared the draft; D.N. Yusuf and Agustono prepared the draft; Asniah and M. Botek reviewed and finalized the draft.

#### **Declaration of Competing Interest**

The authors declare no conflict of interest.

#### **Data Availability**

The data is available from the corresponding author upon request.

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