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The Appearance Of Cocoa Clones Tolerant To Infection *Phytophthora palmivora*

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ABSTRACT

Cocoa pod rot (PBK) is the main disease that attacks cocoa pods caused by *P. palmivora* . Fruit rot disease causes loss of cocoa yield. Cocoa pod rot disease caused by *P. palmivora* pathogen is not a good and stable control method. One strategy that can be used for control is to develop superior clones tolerant of *P. palmivora* . Breeding program to obtain *P. palmivora* tolerant cocoa clones by utilizing cocoa genetic resources derived from potential cultivated clones through detached pot test. The study aimed to obtain cocoa pods tolerant of pod rot using the detached pod test. The research was conducted at the Phytopathology Laboratory of the Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University. The tested cocoa clones consisted of 6 clones, e.g., Sca 12, TSH 858, ICS 60, GC 7, K TSH 858, and K ICS 60. Cocoa clones were collected from a cocoa plantation owned by PT. Host Sari Lubuk Basuk-Agam Regency. The observed variables related to *P. palmivora* tolerant cocoa clones were; incubation period, growth of cacao fruit spot area, and color of cocoa beans. The results showed that the TSH 858 and ICS 60 clones had an incubation period of 3 DAI, while the Sca 12, ICS 60, GC 7, K TSH 858, and K ICS 60 clones had an incubation period of 2 DAI. The TSH 858 clone grew 10.82 cm2.day 1, while the ICS 60, GC 7, K. TSH 858 and K. ICS 60 clones grew in spot area, respectively; 11.42, 12.95, 13.24 and 13.58 cm2.day-1. TSH 858 clone has potential as a tolerant clone to *P. palmivora* attack through detached pod test on fruit.

Keywords: *spot, fruit, detached pot, infection, P. palmivora*

1. INTRODUCTION

Cocoa is a plantation crop, including one of Indonesia's primary export commodities, in addition to oil palm and rubber. Indonesia is the world's third-largest cocoa-producing country after Ivory Coast and Ghana. The total area of cocoa land in Indonesia reaches 1.95 million ha, but Indonesia's productivity is still low at 0.66 $t.ha^{-1}$ compared to cocoa-producing countries such as Malaysia, which reaches 1.8 $t.ha^{-1}$, Ivory Coast at 0.80 $t.\ .ha^{-1}$, and Ghana only reached 0.36 $t.ha^{-1}$ (Sukorini et al., 2021).

Areas included as cocoa development areas in Indonesia include; mostly Sulawesi, which includes; South Sulawesi, West Sulawesi, Southeast Sulawesi, and Central Sulawesi. Sumatra includes Lampung, West Sumatra, North Sumatra, and Aceh, and the island of Java is in East Java and Papua. When viewed from the production of cocoa beans in the period 2018 - 2020 or an average of 740.91 t.yr-1 dry beans, however, from the land area and national cocoa production in the same period, it has decreased. Sulawesi is the region that contributes the most to the national cocoa production, which reaches 70% (Badan Pusat Statistika, 2021).

In addition to the reduced area for cocoa plants, the low productivity of cocoa and various obstacles to cultivating cocoa plants are faced. The main obstacles faced are pests and diseases. The fungus Phytophthora sp is one of the pathogens that attack many plants, which has a broad adverse impact on cultivated plants (Yang et al., 2017; Jung et al., 2018; Anandaraj et al., 2020). The fungus Phytophthora sp has genetic diversity, but seven have been identified as causing diseases, such as cocoa pod rot caused by *P. palmivora* . *P. palmivora* is one of the essential pathogens in cocoa cultivation that must be adequately controlled. *P. palmivora* , in addition to causing fruit rot, also causes canker disease in stems and leaf blight in the seedling stage and mature plants. (Ali et al., 2016; Marelli et al., 2019).

Diseases caused by the pathogen *P. palmivora* on infected cocoa pods show symptoms of necrotic formation from spots that form on leaves, fruit surfaces, and the next stage of cocoa bean rot. This results in decreased production and quality of cocoa beans (Rodríguez-Polanco et al., 2020). The spread of spots was quite fast on the fruit due to the *P. palmivora* attack; within a few days, the entire surface of the fruit and cocoa beans became rotten. *P. palmivora* , as a pathogen, attacks at various stages of cocoa plant growth which can cause the death of cocoa plants up to 10% every year (Nawfetrias et al., (2016). The fungus *P. palmivora* is mycelium and hyphae, which are not septic, with many branches and stiff branches. Cocoa pod rot disease can reduce production from 32.6% to 99%. *P. palmivora* can spread widely and has a high diversity of pathogens that can threaten the decline in cocoa production. *P. palmivora* attacks vary in intensity in different countries. In Peninsular Malaysia, the intensity reaches more than 10%. It reaches 80% to 90% in Cameroon, and disease losses reduce yields from 33% to 50% in Java (Nidhina et al., 2016). Symptoms and levels of pathogenicity caused by *P. palmivora* are strongly influenced by Phytophthora species, cocoa plant genotype, and climatic factors such as humidity, temperature, and rainfall (Puig et al., 2018). According to de Oliviera (2016), the variability of aggressiveness and pathogenicity of *P. palmivora* directly affects infection and disease severity.

The strategy for controlling *P. palmivora* pathogens against cocoa pod rot disease can be done chemically using pesticides. As pathogen control for the long term, pesticides are less friendly to the environment and living things. Control can also be done by biological, cultural methods, genetic resistance, and phytosanitary measures (Acebo-Guerrero et al., 2012). An effective and sustainable control strategy is carried out by developing plant genetic resistance. Control strategies are more economically profitable and, of course, can be combined with other controls. Cocoa plants tolerant to *P. palmivora* are still minimal, and on the other hand, there are also limited potential genetic resources needed in breeding programs. According to (Lessa et al., 2020), Breeding related to plant tolerance to disease is generally considered a complex problem because the effectiveness of resistance is inconsistent and is influenced by the genetic variability of pathogens and host characteristics. According to (Anita-Sari & Susilo, 2014), the development of resistant or tolerant cocoa clones of *P. palmivora* was carried out by tracing potential clones from various germplasm. Agustiawan et al. (2021) explained that MCC 02 is a potential clone tolerant to *P. palmivora* from 4 cocoa clones tested. Due to the limited number of clones produced, it is necessary to test many cultivated cocoa clones that have adapted well to environmental factors.

Efforts to obtain cocoa clones tolerant of *P. palmivora* are essential to testing the existing collection of cocoa clones. One of the cocoa development centers in West Sumatra is PT. Inang Sari has a collection of potential cocoa clones to be developed related to *P. palmivora* tolerance. The potential of cocoa clones that are tolerant of *P. palmivora* infection can be carried out on cocoa pods directly in the field or in the laboratory. The detached pod test (detached pod assay) is a test using fruit related to cocoa resistance carried out in the laboratory, while the attached pod test (attached pod assay) is a cocoa resistance test that is directly carried out on cocoa pods in the field. Testing with the attached pod test method has several obstacles, including; being influenced by climate, including temperature, the humidity of the air around the canopy, rainfall, and a reasonably large area. The detached pod test is one method of tolerance level of cocoa clones to fruit rot disease caused by *P. palmivora* .

The study aimed to obtain cocoa clones tolerant to fruit rot disease caused by *P. palmivora* pathogen infection, using the detached pod test on cocoa pods.

2. RESEARCH METHOD

The research was carried out in the Phytopathology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University, Padang, from February to March 2021.

The materials used are; Healthy cocoa pods aged four months after anthesis (criteria; fruit is fully formed and immature), cocoa is infected with fruit rot, consisting of 6 cocoa clones (Sca 12, TSH 858, ICS 60, GC 7, K TSH 858 and K ICS 60), aqua dest, 70% alcohol, HVS paper, PDA (Potato Dextrose Agar), the equipment used is; petridist dish, cotton, tweezers, oasis needle, object-glass, laminar air flow, hot plate, Scot bottle, beaker, stirrer, knife, engkas, autoclave, binocular microscope, digital camera, and stationery.

The design used was a completely randomized design (CRD) with one treatment in the form of *P. palmivora* mushroom inoculum inoculated on six cocoa clones, consisting of; Sca 12, TSH 858, ICS 60, GC 7, K TSH 858, and K ICS 60.

The cocoa tested was a clone that was collected and cultivated by PT. Inang Sari, which is located in Agam Regency. Cocoa was collected from clones infected with fruit rot disease. *P. palmivora* was isolated from cocoa pods infected by *P. palmivora* , using the method carried out by Rubiyo et al. (2018) as follows; Healthy cacao pods were washed with running water and continued by taking pieces of fruit between the healthy cocoa pods and the infected pods of 5 mm. Fruit pieces were sterilized in 70% alcohol solution for 30 seconds, then dipped in distilled water. Breeding was done by placing the fruit pieces on the growth medium of Potato Destructive Agar (PDA), then incubated for seven days in a dark room with a temperature of 26□C. Mushrooms grown to obtain pure cultures were grown again on the PDA medium. Colonies from the obtained mycelium were then used for further testing.

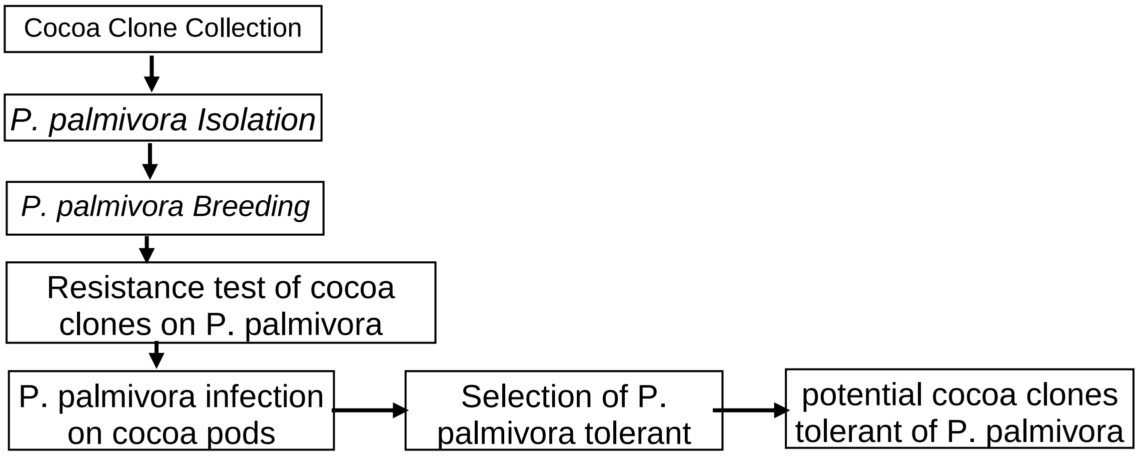

Figure 1. Flowchart of research activities testing several cocoa clones on *P. palmivora* pathogen pathogens

The observed variables include; incubation period, symptom development of *P. palmivora* infection based on incubation period, the spread of spots, and category of *P. palmivora* tolerance cocoa clones. Measuring the area of the spot using the formula;

L = 3,14*(P+l)/4)2

Remark :

L = Spot area P = Spot Length L = Spot Width

The increase in the area of the spots formed is calculated using the formula :

ΔL= (Xn-X(n-1))/N.

Remark:

Xn = Average spot area on day-n

Xn-1 = Average spot area on day n-i

N = Total observations made

Cocoa clone resistance categories are grouped based on the distribution of spots (Rubiyo et al., 2010). Immune, if not showing signs of infection; hold (< 25 cm^2); moderately resistant (25 - 50 cm2); slightly sensitive (50 - 75 cm^2); sensitive ((75 - 100 cm^2); and very sensitive > 100 cm^2.

3. RESULT AND DISCUSSION

Incubation period and spot area

Incubation period and fruit spot distribution of cocoa clones inoculated with *P. palmivora* mycelia using the detached pot assay method. The mass and distribution of spots on the cocoa pod skin three days after inoculation are presented in Table 1.

Table 1. Incubation period and fruit spot growth in cocoa clones inoculated with *P. palmivora* mycelia 2 - 3 days after inoculation (DAI)

Cacao Clone	Incubation Time (DAI)	Spot area 3 DAI (cm^2)
Sca 12	2	4,05
TSH 858	3	3,89
ICS 60	3	3,47
GC 7	2	5,23
K. ICS 60	2	4,46
K. TSH 858	2	4,15
Rata-rata	2	4,21

Table 1 shows that the incubation period of cocoa pods inoculated with *P. palmivora* mycelia ranged from 2 to 3, with an average spot area of 4.21 cm^2. Clones TSH 858 and ICS 60 had an incubation period of 3 DAI, and the incubation period was slower than clones Sca 12, GC 7, K. TSH 858, and K. ICS 60 with an incubation period of 2 DAI. The incubation period was different from the tested cocoa clones, and this was because the cocoa clones had different abilities to defend themselves due to P. palimivora infection before and after penetration. The incubation period also correlated with the area of the spots, clones ICS 60 and TSH 858. besides the long incubation period, the area of the spots formed was 3.47 and 3.89 cm^2, respectively. The incubation period indicates the pathogen's ability to infect plant organs such as fruit and other plant organs. Referring to Adhni et al. (2022), the incubation period is the time required for the pathogen to infect the fruit, which is calculated from the start of the inoculation until symptoms appear on the fruit. The short incubation period of the plant is less resistant to pathogens, so mycelia form more quickly on the fruit. A long incubation period indicates better plant resistance, such as the TSH 858 and ICS 60. clones.

In addition to the incubation period, which is an indicator of resistance to *P. palmivora* infection, the resistance of cocoa clones can be seen from the area of spots formed during the incubation period. During the incubation period of 3 DAI, clones TSH 858 and ICS 60 had a smaller spot area of 3.89 and 3.47 cm^2, respectively. Clones GC 7, K ICS 60, K TSH 858, and Sca 12 were wider at 5.23, 4.46, 4.15, and 4.05 cm^2, respectively. Spot area is one indicator to test the ability of *P. palmivora* to infect cocoa pods. Referring to (Aminullah et al., 2018), the mechanism of plant resistance occurs through several strategies, including reducing the number and area of infected cells or tissues so that it slows down the spread of the fruit skin, suppresses the formation and disposition of spores, as well as of course by increasing the incubation period. This is strongly influenced by the characteristics of the cocoa pods of each tested clone. The wider the spots formed on the surface of the cocoa pods, the lower the resistance level of the cocoa clones. The area of different spots of the tested clones was influenced by the physical and chemical properties that compose the cocoa pod skin. Physically, cocoa pods have differences in skin thickness and toughness. Besides that, cocoa pods chemically contain compounds such as lignin, pectin, and tannins that affect the ability to infect and spread the spots on the cocoa pods widely.

The resistance of the tested cocoa clones related to infection with pathogens such as *P. palmivora* was different based on the incubation period, which was influenced by the shape and structure of the infected fruit. Referring to Soesilo et al. (2015), the characteristics of the fruit that affect the resistance of cocoa plants to diseases include the thickness of the fruit skin, the content of lignin compounds in the fruit skin, and the surface of the fruit skin. One of the critical characteristics of cocoa pods is that they have a skin surface with deep and shallow grooves. Besides that, it also has a rough and slippery surface. Fruit skin with a rough surface efficiently retains water compared to a smooth skin surface so that P. palimivora inoculated on the fruit surface will germinate faster than fruit with a smooth skin surface. The incubation period and distribution of fruit spots formed on cocoa clones infected with *P. palmivora* are shown in Figure 1.

Figure 2. The area of the spots formed on the skin surface of cocoa clones due to *P. palmivora* infection observed during the incubation period of 3 DAI

Figure 2 shows the area of spots formed on five clones due to *P. palmivora* infection as measured by an incubation period of 3 DAI. The area of spots formed from the tested clones, TSH 858 and ICS 60 clones, was smaller than the others, followed by Sca 12, K TSH 858, and GC 7 clones. palmivora, based on the visualization of the fruit, shows the anatomy of *P. palmivora* , such as mycelium, which is white on the part of the fruit that gets injured. In addition to the presence of *P. palmivora* mycelium, there was also the spread of infected tissue damage with discoloration of the fruit skin. Referring to Vanegtern et al. (2018); Afriati et al., 2020); Puig et al. (2021), Symptoms of cacao pod rot disease caused by *P. palmivora* include the formation of brown spots on the fruit and the spread of the spots over the entire surface of the fruit, infecting cocoa beans, and can further cause cancer of the stems and branches if infected. Damage to the skin tissue of the cocoa pod causes brown spots. The brown color spreads from the place of origin of infection to the entire surface of the fruit and attacks the seeds.

P. palmivora infection Symptoms

Symptoms of *P. palmivora* infection based on the rate of increase and the percentage of the area of cacao clone spots on the skin for cocoa during the incubation period of 3 to 7 DAI are presented in Table 2.

Table 2. Increase and percentage of spot area on cocoa clones inoculated with the pathogen Phytophthora palmivora incubation period 3 to 7 DAI.

Cacao Clone	Spot area on the day after inoculation (cm^2)			Percentage of spot area at 7 DAI (%)
	3	5	7	
Sca 12	4,05	19,45	49,21	100
TSH 858	3,89	19,62	54,13	75
ICS 60	3,47	17,81	57,14	80
GC 7	5,23	28,60	64,75	100
K. ICS 60	4,15	24,78	66,21	97
K. TSH 858	4,46	25,57	67,94	98

Table 2 shows the increase in the area of spots formed due to *P. palmivora* infection on the cocoa clones tested (Sca 12, TSH 858, ICS 60, GC 7, K. ICS 60, and K. TSH 858) at 3 DAI was relatively the same. Ranged from 3.47 to 4.46 cm^2. The K ICS 60 and K TSH 858 clones were 4.15 and 4.46 cm wide, followed by the Sca 12 clones with a 4.05 cm^2 area, while the ICS 60 and TSH 838 clones formed spots, respectively. They are 3.47 and 3.89 $cm^{2,}$ respectively. The GC 7 clone had a blot area of 5.23 cm^2. The area of the spots formed at 5 DAI ranged from 17.81 cm^2 to 28.60 cm^2. The area of the spot that was quite large was the GC7 clone reaching 28.00 cm^2, while the smallest was the ICS 60 clone reaching 17.81 cm^2. The increase in spot area up to 7 DAI, TSH 858 clones tended to be slower than ICS 60.

The increase in the area of the spots causes a blackish brown color as a symptom of a pathogen attack covering the cocoa pod skin. TSH 858 and ICS 60 clones at 7 DAI slightly increased the area of the spots, with the percentage of fruit covered by the spots formed being 75 and 80%, respectively. The spread of fruit skin spots increased by clones K ICS 60 and K TSH 858 with the percentage of fruit cover. They were 97 and 98%, respectively. The clones of Sca 12 and GC 7 were clones that had a wide spread of spots covering the entire surface of the fruit skin (100%).

Clones GC 7 and Sca 12 at the age of 7 DAI, the area of spots due to *P. palmivora* infection had covered the entire surface of the skin with a brown color, in contrast to TSH clones 858 and ICS 60 with a smaller percentage of coverage. The pathogenicity of *P. palmivora* was relatively similar to the tested cocoa clones when viewed from the area of the spots produced. This shows that *P. palmivora* , a pathogen, can degrade tissue more quickly, so spreading spots is faster. The percentage of spots that spread due to *P. palmivora* infection in cocoa clones formed three groups, i.e., Sca 12 and GC 7 clones, TSH clones 858 and ICS 60, and K TSH clones 858 with K ICS 60.

The area of the spread of spots covering the entire cocoa pod up to 7 DAI has differences that can be used to indicate the tolerant level of cocoa clones. Referring to Aminullah et al. (2018), Cocoa clones that were sensitive to infection from the *P. palmivora* pathogen tended to have a faster spot spread rate, so the beans rotted more quickly. Resistant cocoa clones, the slower spread of spots due to slow infection ability and long-lasting pathogens before fruit tissue undergoes necrosis. (Barreto et al. 2015); (and Barreto et al., 2018), in his study, explained that the resistance mechanism of cocoa clones was different against inoculation by *P. palmivora* . The development of symptoms that occur is highly dependent on cocoa pod rot and is highly dependent on the cocoa clones and Phytophthora species involved. The rate of increase in the area of spots formed on the skin of cocoa pods due to *P. palmivora* infection is presented in Figure 2.

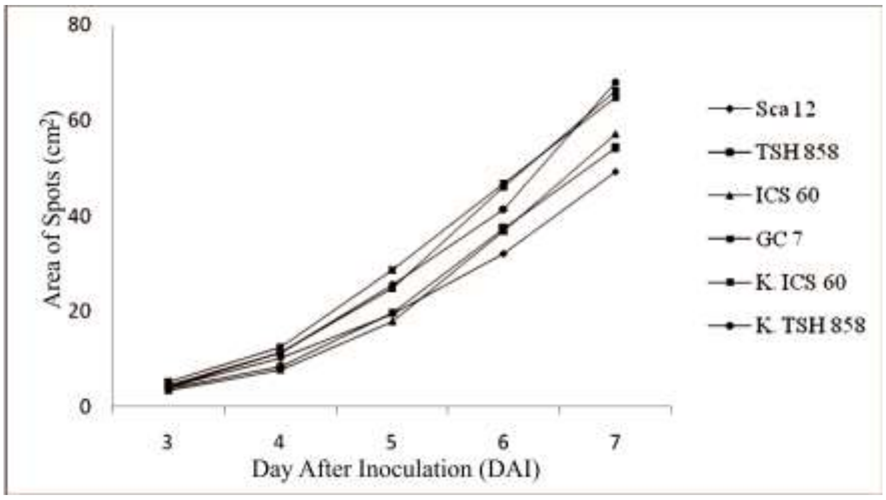

Figure 3. The area of spots on the skin of cocoa clones increased due to *P. palmivora* infection at 3 to 7 DAI.

Figure 3 shows that the increase in the spot area of cocoa clones infected with *P. palmivora* had a difference of up to 7 DAI. The area of the spots formed at 3 and 4 DAI was relatively the same, but at 5, 6, and 7 DAI, there were variations in the rate of spread of the area of the spots in the tested clones. The clones of Sca 12, TSH 858, ICS 60, GC 7, K ICS 60, and K TSH 858 tended to have a faster rate of increase in spot area, with the increase in the area of spots being 9.91, 10.82, 11.42, 12.95, 13.24, respectively. And 13.58 $cm^2.DAI^{-1}$. The spot growth rate of the tested clones started to increase from 5 to 7 DAI, but the Sca 12 clone showed a slower rate of spot growth with a curve that tended to be sloping, but the K TSH 858 clone at 6 and 7 DAI there was an increase in the rate of spot area increase which increased beyond another clone that reaches 67.94 cm^2.

The spread of spots on cocoa clones caused by *P. palmivora* infection correlated with post-penetration. The extent of spots due to damage to the cocoa skin tissue through *P. palmivora* infection is not the initial impact of the infection but becomes a continuation phase after the *P. palmivora* infection phase. The wide distribution of the scab is influenced by internal factors of the cocoa pod skin. Cocoa pods also contain various chemical compounds with chemical defense mechanisms and the role of genetic factors. Chemical compounds found in the fruit's skin affect the spread of spots on the cocoa pods. According to Aminullah et al. (2018), Cocoa skin contains phenolic compounds and tannins. Oxidation of phenolic compounds can produce resistance to disease through inhibitory reactions of pectolytic enzymes and other enzymes. Phenol is formed as a response due to natural damage to plant tissue, which plays a role in repairing damaged tissue. Phenol also inhibits the activity of pectolytic enzymes that play a role in breaking down pectin compounds found in cocoa pods. Pectin in fruit affects the hardness of the fruit skin as an indicator of ripe fruit. In addition to chemically, the spread of spots on the cocoa skin is also strongly influenced by genetic factors. Therefore, the resistance is a plant response that occurs to stress originating from pathogen infection, but each plant has a different response based on the area of the spot. Spots are one of the characteristics of *P. palmivora* 's attack on cocoa pods. According to Merga (2022), the most striking symptom produced by the attack of the pathogen Phytophthora spp. is fruit rot or dark odor. Symptoms on fruit begin as small, firm black spots during the fruit growth. The area of spots on the skin of the cocoa clone fruit that occurred at 7 DAI is presented in Figure 3.

Figure 3. *P. palmivora* pathogen infection with symptoms based on the area of brown spots on the fruit surface, at 7 DAI.

Figure 3 shows that the area of the spots continued to grow until a dark brown color change occurred on the entire surface of the cocoa clones tested. The TSH 858 clone still had a skin on the base and tip of the fruit, while the ICS 60 clone only had the tip of the fruit, and K TSH 858 and K ICS 60 clones were still visible at the thinner end of the fruit. The late end showed a color change in addition to the response of cocoa resistance to different pathogens with an irregular pattern, and the disease attack started from the part of the fruit that was infected with pathogenic mycelia. The color change is one of the symptoms that can be seen in cocoa pods. Mycelia that grow through white inoculation will damage the existing tissue. High pathogenicity and long incubation period are also correlated with the extent of the spread of spots on cocoa pods.

Furthermore, Komalasari et al. (2018) explained that further infection was indicated by the appearance of white powder, a sporangium of *P. palmivora* , on the surface of the infected fruit. According to Vanegtern et al. (2018), sporangium is produced on infected fruit, leaves, stems, or roots. Sporangia can germinate directly on fruit, stems, leaves, and soil and are capable of producing zoospores. Tolerant clones such as clone TSH 858 tended to have a smaller area of distribution than sensitive clones such as clone GC 7.

Cocoa clones grouping due to *P. palmivora* infection

Cocoa clones tested for *P. palmivora* infection on fruit were grouped to determine the nature or category of cocoa plant resistance. Categories based on the area or distribution of the spots formed on the surface of the fruit skin on cocoa clones due to *P. palmivora* infection are presented in Table 3.

Table 3. The area of the spots formed, the increase in the area of the spots, the bean's color, and the category of tolerance level of cocoa clones due to *P. palmivora* infection

Cacao Clone	Increase in spot area (cm^2) at 3 - 7 his	Color seeds on 9 DAI	Area of spot (cm^2) on 7 DAI	Category
Sca 12	9.91	Some Black	49,21	AT
TSH 858	10,82	White	54,13	AP
ICS 60	11,42	Some Black	57,14	AP
GC 7	12,95	Some Black	64,75	AP
K. ICA 60	13,24	Some Black	66,21	AP
K. TSH 858	13,58	Some Black	67,94	AP

Description: AT (somewhat resistant) and AP (somewhat sensitive)

Table 3 shows that, with the increase in spot area, the spot areas are correlated with the tolerance of cocoa clones to pathogens caused by *P. palmivora* . The Sca 12 clone was a clone that was considered moderately resistant with a spot area of 49.21 cm2 or an area of 80% fruit skin cover with a change in seed color to black at 9 DAI. The TSH clone had a spot area of 54.13 cm2, including the moderately sensitive category with 75% fruit skin closure at 9 DAI; the seeds were still white, while the ICS 60, GC 7, K ICA 60, and K TSH 858 clones were categorized as not resistant to seed discoloration goes black at 9 DAI. The TSH 858 clone produced seeds that were still white, and it was suspected that the area of the spots formed was more prominent, but the percentage of fruit cover was smaller than that of Sca 12, so some of the seeds inside the fruit had not been attacked by pathogens. This can be used to indicate that the covering of the fruit with more minor spots can slow down the spread of pathogens in the seeds so that the seeds remain white.

The spreading of spots on cocoa pods infected with *P. palmivora* is also influenced by environmental factors. According to Rubiyo & Amaria (2013), environmental factors influence the development of pathogens in addition to the resistance factor of cocoa clones. Humidity and temperature are factors that affect the growth of pathogens. Mycelium containing sporangium covers the skin of the fruit. The injured fruit no longer has a structure, so the spread of pathogenic infection quickly enters the fruit tissue, so the spread of blackish-brown spots is fast and widespread. The color of cacao clone beans as a result of being infected with *P. palmivora* at 9 DAI is presented in Figure 4.

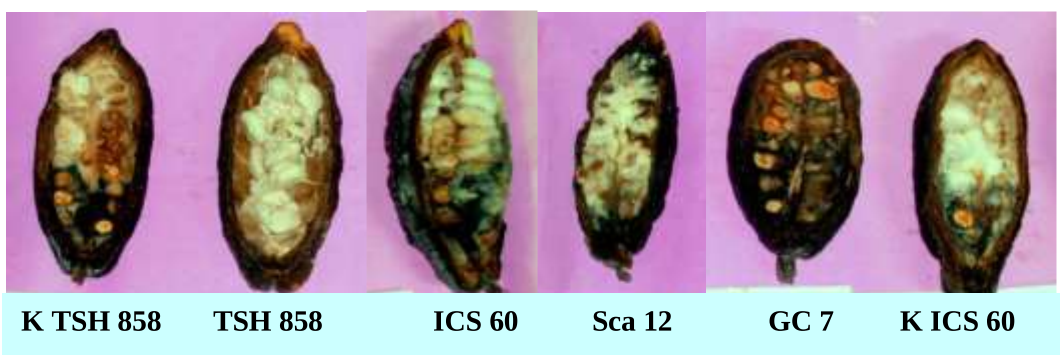

Figure 4. Changes in the color of cocoa clones tested in the laboratory due to *P. palmivora* infection at 9 DAI.

Figure 4 shows that *P. palmivora* infection in cocoa clones, in addition to causing spots on the fruit's skin, also infects the seeds at a later stage. Observing 9 DAI on seeds, clones GC 7 experienced blackish-brown seeds in almost all seeds compared to clones K TSH 858, CS 60, K ICS 60, and SCa 12, while clones TSH 858 seeds were still white. The color change of cocoa beans is highly correlated with the spread of spots on the cocoa husk. Clones that have an area of spots that cover a more comprehensive fruit skin, such as GC7, the area of spots reaches 64.75 cm^2 and even covers the entire surface of the fruit culottes at 9 DAI. The seeds have turned blackish brown. In addition to the TSH 858 clone with an increase in the area of 54.13 cm^2, the spot distribution reached 80%, and the seeds produced were still white, while the other clones changed color to blackish brown due to tissue damage to the fruit. According to (Vanegtern et al., 2018), Symptoms are seen by spreading the spots of the pathogen on the inside of the fruit, including the seeds, infecting and damaging the seeds. Infected seeds and flesh are quickly damaged and rot with discoloration to black and wrinkled. The seeds then dry out and become the primary source of inoculum for infecting fruit, leaves, and stems.

The resistance of cocoa plants to *P. palmivora* pathogens is largely determined by the incubation period. Pathogenic infection of seeds is highly dependent on the incubation period and the speed with which the spots spread on the fruit skin. The long incubation period slows the spread of spots on the cocoa pods. The TSH 858 clone had a long incubation period (3 DAI) with a slower rate of spot growth that inhibited the spread of spots on the fruit skin, so seven of his spots covered 75%. Clones of Sca 12 had a short incubation period with a broader spread of spots on the fruit's skin, which was faster at seven days after the spread of spots had reached 100%, although the area of the spots formed was smaller. This is due to the smaller fruit size, so it is still in the somewhat resistant category.

4. CONCLUSION

The research concluded that the TSH 858 and ICS 60 clones had an incubation period of 3 DAI, while the Sca 12, ICS 60, GC 7, K TSH 858, and K ICS 60 clones had an incubation period of 2 DAI. The TSH 858 clone grew 10.82 cm2.day 1, while the ICS 60, GC 7, K. TSH 858 and K. ICS 60 clones grew in spot area, respectively; 11.42, 12.95, 13.24 and 13.58 $cm^2.day^{-1}$. The TSH 858 clone had the potential as a tolerant clone to *P. palmivora* attack through the detached pod test on the fruit. The tolerance level of cocoa clones needs to be investigated further to get better accuracy by using a genetics-based analysis of cocoa clones.

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