Cocoa Clone Resistance to Phytophthora Pod Rot (PPR) and Cocoa Pod Borer (CPB) in South Sulawesi

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Abstract. This research aimed to find out the resistant of some superior cocoa clones to Phytophthora palmivora in South Sulawesi. This research covers several stages ranging from selection and sampling of cocoa clones, pathogen exploration to cocoa clone’s resistant test with various methods that is attaching the cocoa pod part to the healthy cocoa pod, applying P. palmivora suspension to the cocoa surface and sticking P. Palmivora isolates to the healthy cocoa pod section. The results showed that the resistance of cocoa clones to the three inoculation methods showed different areas of spotting on each observation day. The three application methods show that different applications can affect the difference in infection rates in each cocoa pod clone so that the spots that arise from infection can occur in various ways.

1. Introduction
Cocoa is one of the mainstay of plantation commodities whose role is quite important for the national economy, especially as a provider of employment, sources of income and foreign exchange. Besides that cocoa also plays a role in encouraging regional development and agro-industry. The area of Indonesian cocoa plantations is recorded at 1.7 million hectares. In South Sulawesi the area of cocoa cultivation is recorded at 245,618 ha consisting of smallholders 241,553 ha, large private plantations 4,065 ha, with a total production of approximately 115,326 tons/year [1].

Cocoa development efforts often experience various obstacles, especially by attacks of pests and diseases. One of the diseases that often attacks cocoa is fruit rot caused by Phytophthora palmivora. This pathogen not only causes fruit rot but also stem cancer and leaf blight. The existence of these pathogens can disrupt the growth and development of cocoa plants and can even reduce production. Fruit rot caused by P. palmivora shows symptoms of attack in the form of brownish black spots that start from the base of the fruit and then spread to cover the entire surface of the fruit with whitish gray. The development of patches is quite fast, so that within a few days the entire surface and contents of the fruit become rotten. When the fruit is opened, it will show that the flesh has rot and is black and the seeds are damaged. These fungi have mycelium and hyphae that are not septic, have many branches and are stiff [2], [3].

The control of P. palmivora which is mostly done by farmers is spraying fungicides. However, controlling using excessive synthetic fungicides can have a negative impact on health, environmental
pollution and ecological balance disorders. Therefore, greater attention to biological control. One of the most recommended controls is the use of resistant plants, because these controls are more economically profitable and certainly can be combined with other controls. But the use of resistant plants has several obstacles including the availability of plant material. In addition, planting resistant plants simultaneously can trigger pressure for selection of plant pest organisms (PPO) [4], [5].

In South Sulawesi several superior clones that have high production have been found and developed, including: Sulawesi 1 (S1), GTB, Muchtar 01 (M01), Muchtar 04 (M04), MCC 02 (M 45). However, its resistance to Phytophthora pod rot has not been scientifically confirmed, so research is important to determine the resistance of these clones to *P. palmivora* [6], [7].

2. Materials and Methods

2.1. Cocoa Sampling
Selection and sampling of cocoa was carried out on plantations belonging to residents in the village of Loe, Gantaran Keke sub-district, Bantaeng district, cacao used were 5 types of Sulawesi cocoa clones 1, GTB, M01, M04, and 45. The fruit taken is fruit that is between 3-4 months old which is estimated to have developed well but not yet ripe fruit, and does not have wounds on the surface of the fruit. Then the fruit is brought to the laboratory to be cleaned of dust and dirt that sticks to the surface of the fruit.

2.2. Media preparation
Media used to grow *P. palmivora* fungus is V8 mod juice media. The V8 mod media is made of one made from 100 ml V8 Mod, 900 ml of aquades, 1 gr CaCO3, chloramphenicol, and 17 gr so that [8]. How to make: strain V8 mod juice to be free of dirt. Then mix all ingredients into the Erlenmeyer tube and stir until smooth. After that, close the Erlenmeyer tube using aluminum foil and sterilize it with an autoclave at 121°C for 20 minutes.

2.3. Propagation of *P. palmivora* isolates
*P. palmivora* isolate used was obtained from the Tutik Kuswinanti and Nur Hadina collections found in the Disease laboratory, Department of Plant Pests and Diseases, Hasanuddin University. Isolates are propagated by growing into V8 Mod media. After that, the petri dishes are stored in dark conditions for 7 days.

2.4. Preparation of suspension of *P. palmivora* isolates
*P. palmivora* isolate which has been grown in V8 Mod media, put into erlenmeyer containing 100 ml of aquades and homogenized for 24 hours. The suspension solution of *P. palmivora* was taken as much as 10 ml then calculated the amount of spores using a haemocytometer using a microscope. Spore concentration is calculated using the formula:

\[
K = \frac{t}{N} \times 0.25 \times 10^6
\]

Note:
- \( K \) = Spore density / spore concentration
- \( t \) = the average number of spores in the box observed
- \( N \) = The total number of boxes
2.5. **Procedure**

The cocoa fruit used is 3 months old cocoa fruit from 5 existing clones taken from the field to be tested in the laboratory. Before the fruit is applied, the fruit is cleaned first by washing it in running water, after that the fruit is cleaned by spraying 70% alcohol on the fruit after that, dry it and store it in a clean box then save it.

2.5.1. **Method I (Attaching the sick fruit to the healthy fruit section).** Healthy cocoa fruit is injured by making a hole with a diameter of 9 mm and a depth of 5 mm. After that the sick cocoa fruit is taken in the middle part of the symptom of infection (50% of the sick part, 50% of the healthy part) of the same size in healthy fruit. The part is inoculated on healthy fruit and after that the fruit is wrapped in a plastic warp to avoid contamination and keep the moisture and stored in a dark place at room temperature for 6 days.

2.5.2. **Method II (Apply P. palmivora solution to the surface of the cocoa fruit).** Making a suspension of *P. palmivora* in a ratio of 100 ml of sterile aquades added 2 plates of *P. palmivora* isolates. Then homogenize for 24 hours. Healthy fruit that has been sterilized is then applied with a suspension of *P. palmivora*. After that the fruit is stored in a box that has been sterilized and covered with plastic warp to avoid contamination and maintain moisture. The box is stored in a dark place with room temperature.

2.5.3. **Method III (Attaching P. palmivora isolates to healthy fruit section).** Healthy cocoa fruit is injured by making a hole with a diameter of 9 mm and a depth of 5 mm. After that cut the V8 Mod Juice medium of the same size 9 mm with the part of *P. palmivora* mycelia. After that the media is inoculated on healthy fruit and after that the fruit is wrapped in a plastic warp to avoid contamination and keep the moisture and stored in a dark place at room temperature for 6 days.

After that, the observations made by measuring the length and width of the spots on the surface of the fruit, then the measurement results are inserted into the following formula to calculate the area of spots on the surface of the cocoa:

\[
L = \frac{(p + l)^2}{4} 
\]

Note:
- \(L\) = Area of Spots
- \(p\) = length
- \(l\) = width

3. **Results**

From testing the resistance of several cocoa clones to *P. palmivora* infection, the results of the cocoa fruit test using the symptomatic method of attaching the fruit directly, attaching *P. palmivora* isolates grown in V8 Mod media and applying *P. palmivora* suspension to the fruit surface, showing all clones that have been tested have symptoms of fruit rot. This is indicated by the appearance of brown spots around the inoculated part and the white mushroom mycelia on the surface of the fruit. Fruit infected with *P. palmivora* will cause the surface of the fruit to be brownish-black and sufficient moisture to cause the entire surface of the fruit to be filled with white to gray mycelia.
3.1. Average growth of spots
Observation of the average area of spots on cocoa fruit until the last day of observation is presented in Table 1, Table 2 and Table 3. From the data obtained the cacao fruits of all the clones tested belonging to the group "very vulnerable" to the harming of fruit. This is indicated by the average total area of the spotted fruit on the last observation day after inoculation, which is more than 100 cm².

Table 1. Extent of spotting on cocoa clones inoculated with *P. palmivora* fungus by attaching symptomatic fruit parts to the surface of healthy fruit

| Cocoa Clone | Area of Spots on Observation Day (cm²) | Average Increase of Spots (cm²/day) |
|-------------|----------------------------------------|-----------------------------------|
|             | 2 | 3 | 4 | 5 | 6 |                |
| 45          | 12.69 | 43.57 | 133.66 | 275.52 | 398.36 | 79.67 |
| GTB         | 28.91 | 65.31 | 104.49 | 153.20 | 233.22 | 46.64 |
| MO1         | 9.68 | 38.88 | 86.45 | 137.86 | 231.71 | 46.34 |
| MO4         | 22.01 | 57.61 | 98.32 | 160.10 | 271.43 | 54.29 |
| S1          | 16.72 | 33.82 | 71.22 | 141.30 | 234.83 | 46.97 |

Description: The average spotting area (ΔL) is calculated by the formula ΔL = Σ (Xn - X (n-1)) / N, Xn is the average spotting area on day-n and X (n-1) is the average spotting area on day n-1, N is the number of observations made.

Based on Table 1. Of all the cocoa clones that were tested on average the increase in spotting per day showed the development of *P. palmivora*. The slowest growth occurred in the MO1 cocoa clone (46.34 cm²/day). While the largest increase in patches per day is shown in 45 cacao clones (79.67 cm²/day).

Table 2. Addition of patch spots on cocoa clones inoculated with *P. palmivora* fungi by applying *P. palmivora* suspension to fruit surface

| Cocoa Clone | Area of Spots on Observation Day (cm²) | Average Increase of Spots (cm²/day) |
|-------------|----------------------------------------|-----------------------------------|
|             | 2 | 3 | 4 | 5 | 6 | 7 |                |
| 45          | 0.00 | 25.38 | 39.85 | 68.02 | 91.82 | 121.91 | 18.36 |
| GTB         | 0.00 | 0.00 | 1.72 | 9.66 | 50.56 | 124.04 | 10.11 |
| MO1         | 10.41 | 18.84 | 32.57 | 64.87 | 125.93 | 237.12 | 25.19 |
| MO4         | 0.00 | 11.76 | 22.71 | 28.94 | 73.31 | 115.24 | 14.66 |
| S1          | 0.00 | 6.41 | 10.22 | 19.25 | 25.88 | 30.43 | 5.18 |

Description: The average spotting area (ΔL) is calculated by the formula ΔL = Σ (Xn - X (n-1)) / N, Xn is the average spotting area on day-n and X (n-1) is the average spotting area on day n-1, N is the number of observations made.

Based on Table 2, the average area of spotting per day shows the development of *P. palmivora*. The slowest growth occurs in S1 cocoa clones (5.18 cm²/day). Whereas the largest area of spotting per day is shown in the MO1 cocoa clone (25.19 cm²/day).
Table 3. Addition of patch spots on cocoa clones inoculated with *P. palmivora* fungus by attaching *P. palmivora* isolates grown in V8 mod media

| cocoa clone | Area of Spots on Observation Day (cm²) | Average Increase of Spots (cm²/day) |
|-------------|----------------------------------------|-------------------------------------|
| 45          | 1.35 8.50 22.95 66.70 82.72 122.57     | 16.54                               |
| GTB         | 0.00 2.34 9.56 27.95 76.41 103.50       | 15.28                               |
| MO1         | 0.00 6.44 22.27 39.95 82.16 125.93      | 16.43                               |
| MO4         | 2.23 14.27 36.84 84.60 111.40 165.01    | 22.28                               |
| S1          | 2.88 13.98 33.89 65.14 90.52 129.27     | 18.10                               |

Description: The average spotting area (ΔL) is calculated by the formula ΔL = Σ (Xn-X (n-1)) / N, Xn is the average spotting area on nth day and X (n-1) is the average spotting area on day n-1, N is the number of observations made.

Based on table 3, the average area of spotting per day shows the development of *P. palmivora*. The slowest growth occurred in GTB cocoa clones (15.28 cm²/day). Whereas the largest area of spotting per day is shown in MO4 cocoa clones (22.28 cm²/day).

3.2. Relation between area of spots with the level of cocoa clone resistance

Table 4. Spots and response of cocoa fruit to infection of *Phytophthora palmivora* after inoculation with several methods to the surface of healthy fruit.

| Cocoa Clone | Area of Spots (cm²/day) After being inoculated into healthy fruit |
|-------------|---------------------------------------------------------------|
|             | Method 1 | Method 2 | Method 3 |
|             | 6th day | Response | 7th day | Response | 7th day | Response |
| 45          | 398.36  | SR       | 121.91  | SR       | 122.57  | SR       |
| GTB         | 233.22  | SR       | 124.04  | SR       | 103.50  | SR       |
| MO1         | 231.71  | SR       | 237.12  | SR       | 125.93  | SR       |
| MO4         | 271.43  | SR       | 115.24  | SR       | 165.01  | SR       |
| S1          | 234.83  | SR       | 30.43   | AT       | 129.27  | SR       |

Description: * TH: resistant (spotting area <25 cm²), AT: somewhat resistant (25-50cm²), AR: somewhat vulnerable, RT: vulnerable (25-50 cm²), and SR: very vulnerable (> 100 cm²) to *P. palmivora* infection.

From the results of this observation, it was shown that in method 1 and 3 cocoa clones 45, GTB, MO1 and MO4 had a very susceptible response to cacao fruit rot infection, because the average spotting area on the last day of observation was above 100 cm². Whereas in the second method S1 cocoa clones that had a rather resistant response were compared with other cocoa clones because the spotting area on the last day observation was below 50 cm².

4. Discussion

Observation of spotting broadness in the three methods used showed the development of the area of patches on cocoa clones inoculated with *P. palmivora* fungus with Method 1 which is attaching symptomatic fruit to the surface of healthy fruit (Table 1) shows that the slowest growth occurred in the cocoa MO1 clones, against infection with rotten diseases compared to other clones. Method 2 The addition of patches on cocoa clones by applying a suspension of *P. palmivora* to the fruit surface
(Table 2) shows that S1 cocoa clones tend to be more resistant to infection with fruit rot than other clones. As well as method 3 (Table 3), which is attaching *P. palmivora* isolates grown on V8 mod media, the slowest growth occurred in GTB cocoa clones, this indicates that GTB cocoa clones tend to be more resistant to infection with fruit rot. This might be triggered by the resistance of cocoa fruit clones which are influenced by the morphological nature of the cocoa fruit itself, but it is also suspected because the tendency of fruit resistance also correlates with other resistance mechanisms [9].

Addition of patches on cocoa clones by Method 1 showed the slowest growth occurred in the MO1 cocoa clone with an average growth of patches of 46.34 cm²/day and the highest was shown in 45 cocoa clones of 79.67 cm²/day. Method 2 shows that the slowest growth occurs in S1 cocoa clones which are 5.18 cm²/day and the largest at MO1 25.19 cm²/day. And in Method 3 the slowest growth occurred in GTB cocoa clones (15.28 cm²/day) and the largest was shown in M04 cocoa clones (22.28 cm²/day). From the results above it can be seen that in method 1 the growth of MO1 clones was the slowest but in method 2 the growth of MO1 clones was greatest.

This shows that the way in which different plant pathogens enter results in different levels of infection. The way in which pathogens enter different plants can trigger plant resistance responses that vary depending on the type of clone. This is in accordance with the opinion of Marinus "the better the resistance of plants after penetration, the incubation period will be longer and the development of spots will be slow, and vice versa if the plant is not able to inhibit pathogens into the network, the plants will be easily infected by pathogens, incubation will be faster and produce larger spots ".

The relationship of the spotting area with the resistance level of cocoa fruit from the three application methods that in method 1 and 3 cocoa clones 45, GTB, MO1 and M04 had a very susceptible response to cocoa fruit rot infection, because the average spotting area on the last observation day was above 100 cm². Whereas in the second method S1 cocoa clones that had a rather resistant response were compared with other cocoa clones because the spotting area on the last day observation was below 50 cm². This shows that the six-day symptom fruiting method showed values> 100 cm² in all cacao clones observed even > 200 cm² which indicated that each clone was very susceptible to *P. palmivora* infection by method 1. For method 2, by applying the *P. palmivora* suspension to the surface of the fruit, it appears that on the seventh day 45, GTB, MO1 and M04 clones showed *P. palmivora* infection> 100 cm² which indicated that each clone was very susceptible, except for S1 clones which were rather resistant (25-50 cm²) with spotting area of 30.43 cm²/day. As well as method 3 by attaching *P. palmivora* isolates grown on V8 mod media, showing values > 100 cm² on all observed cocoa clones which indicated that each clone was very susceptible to *P. palmivora* infection. Spotting area can be the main parameter of cocoa fruit resistance to pathogenic infections that cause fruit rot.

Structural defense includes the number and quality of candles and cuticles that cover epidermal cells, the size, location and shape of the stomata and lenticel, and the thick tissue of cell walls that inhibits pathogen progression. The compounds produced by plant tissue before an attack of pathogens are phenolic and tannin. Phenolic compounds and their oxidation results can produce disease resistance through inhibition of pectolytic enzymes and other pathogenic enzymes. This can affect the difference in infection rates in each method so that the spots that occur due to infection can occur in a variety of ways.

5. Conclusion

Cocoa clone resistance from the three inoculation methods showed different spotting areas on each observation day which in method 1 showed the highest spotting area, namely on the 45 clones with a value of 398.36 cm² on day 6 with an average spotting increase of 79.67 cm²/day. All the clones tested all had a very susceptible response to resistance except for the S1 clone in method 2 with a rather resistant response. Different application methods can affect the difference in infection rates in each cocoa clone so that the spots that occur due to infection can occur in a variety of ways.
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