Resistance test of five cocoa (Theobromae cacao L.) clones in South Sulawesi against Phytophthora palmivora

V S Dewi, A Asman, K Josua and H Hamdayanty

Plant Pest and Disease Department, Faculty of Agriculture, Universitas Hasanuddin, Makassar, 90245, Indonesia.

E-mail: fachrudinvien@yahoo.com

Abstract. This research aimed to find out the response of some superior cocoa clones in South Sulawesi to Phytophthora palmivora. This research covers several stages ranging from selection and sampling of cocoa clones, pathogen exploration to cocoa clones resistant test with various methods that were inoculated the infected to the healthy cocoa pod, inoculated P. palmivora suspension to the cocoa surface and inoculated P. Palmiora isolates to the healthy cocoa pod section. The results showed that the resistance of cocoa clones to three inoculation methods showed different areas of spotting on each observation day in which method of attaching the infected pod to the healthy pod, showed the slowest growth in the MO1 cocoa clone with average growth of spots of 46.34 cm²/day and the largest was shown in 45 cocoa clone which was 79.67 cm²/day indicating each clone is very susceptible to infection P. palmivora. Method of inoculating P. palmivora suspension showed that the slowest growth occurred in S1 cocoa clone that was 5.18 cm²/day and largest was shown in MO1 cocoa clone (25.19 cm²/day), indicating that each clone is very susceptible, except in the clone S1 is rather resistant. As well as in the method of attaching P. palmivora isolate to the healthy pod, the slowest growth occurred in GTB cocoa clones (15.28 cm²/day) and largest in M04 cocoa clones (22.28 cm²/day) which indicated that each clone was very susceptible to infection of P. palmivora. The three application methods show that different applications can affect the difference in infection rates in each cocoa pod clone. The spots that arise from infection can occur in various ways.

1. Introduction
Pod rot caused by Phytophthora palmivora is one of the important diseases in cocoa plants in Indonesia, especially in South Sulawesi. P. palmivora not only causes pod rot but also stem cancer and leaf blight. The existence of these pathogens can disrupt the growth and development of cocoa plants, causes serious production losses in all cocoa growing zones, varying from 20-40% global yield loss and 10% tree deaths annually [1, 2]. Most infections of P. palmivora causes crop death and decrease in production. In most cases, the disease caused by P. palmivora actively spreads during high humidity and wet conditions [3].

Spraying fungicides is method that mostly used by farmer to control P. palmivora. However, excessive control of using synthetic fungicides have a negative impact on health, environmental pollution and ecological balance disorders. Therefore, attention to biological control is greater than others [4]. One of the most recommended controls is using resistant plants. It is an essential ecological and economical solution for integrated and sustainable control [5]. But using resistant plants has
several obstacles including the availability of plant material. In addition, planting resistant plants simultaneously can trigger pressure selection for pests and plant diseases.

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 (M45). 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*.

The purpose of this study was to determine the resistance of several superior cocoa clones to *P. palmivora* found in southern Sulawesi. This research aims to determine the level of resistance of cocoa clones and as a consideration for farmers in determining the cocoa clones to be cultivated.

2. Materials and methods

2.1. Plant material

Selection and sampling of cocoa were carried out at Bantaeng, South Sulawesi. The cocoa clones used were Sulawesi 1, GTB, M01, M04, and 45. The age of pod between 3-4 months, estimated that the pod has developed well but is not yet ripe, and does not have wounds on the surface of the pod.

2.2. *P. palmivora* material

The *P. palmivora* isolate used was obtained from collections of Plant Disease Laboratory, Department of Plant Pests and Diseases, Universitas Hasanuddin. Isolate was propagated to V8 Mod media and stored in dark conditions for 7 days. *P. palmivora* isolate, which has been grown in V8 Mod media, homogenized with 100 mL aquades for 24 hours.

2.3. Cocoa pod inoculations

The 5 clones of the cocoa pod are cleaned in running water, sprayed with 70% alcohol, dried, and stored in the sample box. Inoculations were carried out with 3 methods. (1) Attached the sick pod to the healthy pod section. Healthy pod were injured by making a hole with a diameter of 9 mm and a depth of 5 mm. The pod rot were taken in the middle part of the symptom of infection (50% of the sick part, 50% of the healthy part) and inoculated on healthy pod. (2) Inoculated *P. palmivora* solution to the surface of the healthy pod. *P. palmivora* suspension was made by dissolving 2 plates of *P. palmivora* isolates with 100 mL of distilled water and shaking for 24 hours. Healthy pod that had been sterilized were inoculated with *P. palmivora* suspension. (3) Attached *P. palmivora* isolates to healthy pod. Healthy pod are injured by making a hole with a diameter of 9 mm and a depth of 5 mm. *P. palmivora* isolates on v8 mod juice media (diameter: 9 mm) were inoculated on healthy pod. Each sample were stored in a box that has been sterilized and covered with plastic wrap to avoid contamination and maintain moisture. The boxes were stored in a dark place with room temperature for 6 days.

2.4. Observations

Observations were made by measuring the length and width of spots on the surface of the pod to determine the extent of infection. The resistance of cacao pod to *Phytophthora* sp. grouped based on the area of the spots that appear until the last day of observation according to the classification used by Suhendi et al 2005 [6]. Classification of cocoa pod resistance level to *P. palmivora* infection were TH: spot area <25 cm$^2$ (resistant), AT: spot area 25-50 cm$^2$ (rather resistant), AR: spot area 50-75 cm$^2$ (susceptible), and SR: >100 cm$^2$ (very susceptible). The experiment was arranged in a completely randomized design. Six pods (2 pieces/unit x 3 replications) were inoculated for each cocoa clone. The total cacao clones tested were 5 clones and the total pods used in each clone were 18 pods.
3. Results and Discussion

Based on the 3 inoculation methods carried out on cocoa pod showed that all tested clones had symptoms of pod rot. It was indicated by the appearance of brown spots around the inoculated parts and white mushroom mycelia on the surface of the pod. Pod infected with *P. palmivora* will cause the surface of the pod to be brownish-black. Sufficient humidity caused the entire surface of the pod to be filled with white to gray mycelia.

The average area of spots on cocoa pod until the last day of observation are presented in table 1, table 2 and table 3. The average total area of pod spots was more than 100 cm². Symptomatic observation by method 1 was only carried out until the 6th day because the area of *P. palmivora* infection was more than 200 cm². This shows that the cocoa pods from all clones tested were classified as very susceptible to pod rot, except S1 cocoa clone by inoculating *P. palmivora* suspension to pod surface method.

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

| Cocoa clones | Spotting area on observation day (cm²) | Average increase in infected spot area (cm²/day) |
|--------------|----------------------------------------|-------------------------------------------------|
| 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 98.32 160.01 271.43         | 54.29                                           |
| S1           | 16.72 33.82 71.22 141.30 234.83         | 46.97                                           |

The average increase in infected spot area was calculated by dividing the area of infected area on the sixth day of observation by the number of days of observation.

The *P. palmovira* inoculation method by attaching symptomatic pod parts to the surface of healthy pods showed that *P. palmivora* was able to quickly infect healthy pods (table 1). Symptoms of the disease with a fairly high area of infection have appeared on the second day of observation, reaching 28.91 cm² in GTB clones. The progression of the disease is characterized by the rapid increase in area of infection, which is also high, reaching 79.67 cm²/day in 45 clone. This shows that *P. palmivora* can infect clone 45 quickly using the method of direct attachment of diseased pods to healthy pods. The lowest *P. palmovira* infection with this method is shown in MO1 clone (46.34 cm²/day).

**Table 2.** Addition of patches on cocoa clones inoculated with *P. palmivora* fungi by applying *P. palmivora* suspension to pod surface.

| Cocoa clones | Spotting area on observation day (cm²) | Average increase in infected spot area (cm²/day) |
|--------------|----------------------------------------|-------------------------------------------------|
| 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                                            |

The average increase in infected spot area was calculated by dividing the area of infected area on the sixth day of observation by the number of days of observation.
Based on table 2, the average area of spotting per day indicates the development of *P. palmivora*. The slowest growth occurs in S1 cocoa clones (5.18 cm\(^2\)/day). The largest area of spotting per day is shown in the MO1 cocoa clone (25.19 cm\(^2\)/day). The development of symptoms with this method seems slower than the direct attachment of symptomatic pod to healthy cacao.

From the results above it appears 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 pathogens enter the plant causes different levels of infection. The way in which pathogens enter different plants can trigger a different plant resistance response depending on the type of clone. The higher the plant resistance after penetration, the incubation period will be longer and the development of symptoms will be slow. If plants are not able to inhibit pathogens into the tissue, plants will be easily infected by pathogens, incubation will be faster and produce greater symptoms [7].

**Table 3.** Addition of spots on cocoa clones inoculated with *P. palmivora* fungi by attaching *P. palmivora* isolates grown in V8 mod media.

| Cocoa clones | Spotted area on observation day (cm\(^2\)) | Average increase in infected spot area (cm\(^2\)/day)* |
|--------------|-------------------------------------------|------------------------------------------------------|
|              | 2 | 3 | 4 | 5 | 6 | 7 |
| 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 |

The average increase in infected spot area was calculated by dividing the area of infected area on the sixth day of observation by the number of days of observation.

The slowest growth of *P. palmivora* with method 3, which is attaching *P. palmivora* isolates grown on V8 mod media, occurred in GTB cocoa clones (15.28 cm\(^2\)/day). The largest area of spotting per day was indicated by MO4 cocoa clones (22.28 cm\(^2\)/day) (table 3). Differences in response from each clone based on the inoculation method triggered by the resistance of cocoa pod clones which are influenced by the morphological nature of the cocoa pod itself, but it is also suspected because the tendency of pod resistance also correlates with other resistance mechanisms [8].

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

| Cocoa clones | Spotting area (cm\(^2\)/day) after inoculation to healthy pod |
|--------------|------------------------------------------------------------|
|              | Method 1 | Method 2 | Method 3 |
|              | Day 6 | Response | Day 7 | Response | Day 7 | 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: Classification of cocoa pod resistance level. TH: resistant (spotting area <25 cm\(^2\)), AT: rather resistant (25-50 cm\(^2\)), AR: susceptible (25-50 cm\(^2\)), and SR: very susceptible (>100 cm\(^2\)) to *P. palmivora* infection.
The relationship of the spotting area with the resistance level of the cocoa pod from the three application methods that in method 1 and 3 cocoa clones 45, GTB, M01 and M04 had a very susceptible response to cocoa rot infection, because the average spotting area on the last observation day was above 100 cm². Whereas in the second method, S1 cocoa clones had a rather resistant response were compared with other cocoa clones because the spotting area in the last day observation was below 50 cm². This shows that the method of attaching infected pod at the sixth day observation showed a value of >100 cm² on all cocoa clones observed even >200 cm² at 45 cocoa clone which indicated that each clone was very susceptible to \textit{P. palmivora} infection with method 1. For method 2 by inoculating \textit{P. palmivora} suspension to the pod surface showed \textit{P. palmivora} infection >100 cm² at 45, GTB, MO1 and MO4 clones which indicated that each clone was very susceptible, except for S1 clone which were rather resistant (25-50 cm²) with spotting area 30,43 cm²/day. As well as method 3 by attaching \textit{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 \textit{P. palmivora} infection. Iwaro et al [9] stated that spotting area can be the main parameter of cocoa pod resistance to pathogenic infections that cause pod rot.

Surface of leaves and cacao pods have a primary path that is expected to influence dissemination, disposition and growth of pre-penetration inoculum. Pod shape will affect inoculum disposition, for example the type of cocoa that has rough skin will hold water between the skin of cocoa, so the inoculum will germinate and penetrate on cacao pod [8]. Furthermore Iwaro et al [10] stated that the morphological characteristics of pod were not correlated with post-penetration resistance, this shows possibility the role of biochemical mechanisms.

Structural defense includes the number and quality of candles and cuticles that cover epidermal cells, the size, location and shape of the stomata and lenticels, 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 reactions of pectolytic enzymes and other pathogenic enzymes [7]. This can affect the difference in infection rates in each method so that the spots that occur due to infection can occur in various ways.

4. Conclusions
- Cocoa clones resistance on the three inoculation methods showed different patches area on each observation day, whereas method 1 showed the highest spot area on 45 cocoa clone with a value of 398.36 cm² on the 6th day with an average increase spotting area 79.67 cm²/day
- All of cocoa clones had a very resistance response except for S1 cocoa clone in method 2 with rather resistant responses.
- Different application methods can affect the different levels of infection in each cocoa clone so that patches that arise due to infection can vary.

Reference
[1] Hervé K S, Mireille A W A and Marie-David C N A 2017 A review on cocoa plant diseases and importance of \textit{Bacillus subtilis} to induce resistance on crops in agriculture Indian J. Agric. Res. 53 125-13
[2] Rubiyyo and Amaria W 2013 Ketahanan tanaman kakao terhadap penyakit buah buah (\textit{Phytophthora palmivora} Butl.) Perspektif 12 23–36
[3] McMahon P and Purwantara A 2004 \textit{Phytophthora} on cocoa in major crops affected by \textit{Phytophthora} In Drenth A and Guest D I (Eds.) Diversity and Management of \textit{Phytophthora} in Southeast Asia ACIAR Monograph 114 (Canberra: ACIAR) p 104-115
[4] Harni R, Amaria W, Ferry Y and Marhaeni L S 2020 Effect of \textit{Trichoderma} spp. and potassium fertilizer on \textit{Phytophthora palmivora} infection in cacao seedlings 1st Int. Conf. on Sustain. Plant. IOP Conf. Series: Earth and Environmental Science 418
[5] Amponsah J D 1988 Studies on field resistance of cocoa varieties to *P. palmivora* (Butl.) Butl. infection *Proc. 10th Int. Cocoa 303 Res. Conf. Santo Domingo, Dominican Republic* 1987 557–567

[6] Suhendi D, Winarno H and Susilo A W 2005 Peningkatan produksi dan mutu hasil kakao melalui penggunaan klon baru *Pros. Simp. Kakao Pusat Penelitian Kopi dan Kakao Indonesia* 4

[7] Agrios G N 2005 *Plant Pathology* 5th Edition (USA: Elsevier Academic Press) p 922

[8] Rubiyo, Purwantara A and Sudarsono 2010 Ketahanan 35 klon kakao terhadap infeksi *Phytophthora palmivora* Butl. *J. Nat. Sci.* 3 50-56.

[9] Iwaro A D, Butler D R and Eskes A B 2006 Sources of resistance to *Phytophthora* pod rot at the international cocoa genebank, Trinidad *Genetic Resources and Crop Evolution* 53 99-109

[10] Iwaro D A, Sreenivasan T N and Umaharan 1997 *Phytophthora palmivora* resistance in cocoa (*Theobroma cocoa*): Influence of pod morphological characteristics *Plant Pathology* 46 557-565