Evaluation of thirty Indonesian chili varieties for resistance to *Phythophthora capsici*

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**Abstract.** The disease caused by *P. capsici* can attack all tissue and growth phases of the chili plant with the yield loss up to 100%. The research aimed to evaluate the resistance response of thirty Indonesian chili varieties against *P. capsici* that have been carried out at the IVEGRI research station - Lembang, West Java since April until July 2020. The selection method was carried out using the artificial inoculation. A total of 10 ml of zoospores with a density of \(10^6\) were inoculated on chili seedlings five weeks after sowing. The observations were carried out five times. The observation was carried out at 7 to 35 days after inoculation with intervals of 7 days. Based on disease severity (DS), the resistances of varieties were grouped into three levels, namely resistant (DS<20%), moderately resistant (20%<DS<50%), and susceptible (DS>50%). The result showed variations on symptoms, scoring, and the time of symptom appearance in and between the varieties tested. The evaluation result showed that 24 varieties were resistant, 5 moderately resistant, and 1 susceptible to *P. capsici*.

1. **Introduction**

Chili is one of the essential ingredients for cooking in tropical and sub-tropical countries and the fourth main crop grown globally [1]. The consumption of red chilies (kg) per capita per year in Indonesia from 2015-2019 was 2.97, 2.29, 1.77, 3.54, and 1.98, respectively. Meanwhile, the consumption of green chilies from 2017 - 2019 was fixed at 0.365 [2]. The national chili productivity based on the Ministry of Agriculture [3] is 7.94 tons / ha, but according to Syukur et. al [4] productivity of chili can reach up to 20 tons / ha. There are about 400 different varieties of chili grown in the world. As a cooking spice grown around the world, many limiting factors can reduce production, causing significant reductions in yield and seed production. Plant diseases are the main reason for crop loss in the world [1].

Disease in chili plants caused by *P. capsici* Leonian can attack all tissues and phases of plant growth, from seedling to the generative phase [5]. This pathogenic fungus can also attack the Cucurbitaceae (cucumber, melon, pumpkin, watermelon), Solanaceae (chili, tomato, eggplant), and Leguminosae families (green beans, kratok beans) [6]. Symptoms of the disease caused by *P. capsici* vary, such as...
root rot, stem blight, leaf blight, and fruit rot [7]. Oospora P. capsici can survive for several years in soil and can move through wind and water when temperatures are warm (25-28°C) and when conditions are humid during the rainy season [5].

This fungus is a pathogen that damages chili plants worldwide and causes high yield loss. In Indonesia, this fungus attack has destroyed more than 60% of the chili cultivation area of farmers in Tegal [8]. Meanwhile, the yield loss due to P. capsici attacks in Turkey reached more than 40% [9]. In the cucumber crop, the first reports of yield loss (1937) were as high as 100% [10]. Various cultivation techniques have been used to control this disease, such as not planting in areas with a history of P. capsici infestation, improve irrigation and drainage systems, rotation with less susceptible crops, and fungicides application. However, there has been no effective, practical, economical and safe method to control P. capsici [11]. Bosland and Votava [12] stated that fungicides were not effective in controlling P. capsici which attacked roots. One of the effective ways to control P. capsici is to use resistant varieties. The use of resistant varieties can reduce production costs and reduce the risk due to pesticide residues. Sujiprihati et al. [13] have selected resistant chili varieties against P. capsici, by obtaining 1 resistant genotype, namely PSPT-C11 which is used as the parent gene donor. This study aims to determine the resistance response of 30 local Indonesian chili genotypes to P. capsici.

2. Materials and methods

2.1. Time and study site
The research was carried out from April to July 2020 in greenhouses and the laboratory of the Indonesian Vegetable Crops Research Institute.

2.2. Materials and tools
The materials used were 3 weeks old Indonesian local varieties of chili seeds, inoculum of P. capsici, and PDA media. The tools used are Hemocytometer, microscope, micropipette, tip, measuring cup, scalpel knife, rolling glass.

2.3. P. capsici inoculum preparation
P. capsici inoculums were taken from chili plants with symptoms of stem rot or leaf blight to be isolated. The isolated results were then purified and identified using a microscope. Identification using the Pictorial Atlas of Soil and Seed Fungi book [14].

2.4. Inoculation of P. capsici fungi into chili plants
Inoculation of the fungus P. capsici into chili plants refers to Wartono et al. [15] with several modifications. One-week old P. capsici fungal inoculum was gently scrapped with a scalpel knife and the medium was crushed until smooth using a rolling glass, then 10 mL of sterile distilled water was added. The source of this pure inoculum is then stressed for 2 hours in the refrigerator (temperature 40°C) with the aim of initiating zoospores. The source of the inoculum was removed from the refrigerator, 90 mL of sterile distilled water was added and shaken for 2 hours. Then the spore count was carried out by means of suspension taken as much as 0.1 µl and dropping it on a hemocytometer and observed under a microscope. The spore density used for the application was $5 \times 10^6$. The suspension was added with sterile distilled water up to 1,100 mL to obtain the desired spore density. A total of 10 mL of the suspension was poured on the chili plants, then the plants were watered every day. Observations were made at 7 days after inoculation (dai), 14 dai, 21 dai, 28 dai, and 35 dai. The disease index is calculated by the formula:

$$ DI = \frac{\sum (n_i \times v_i) \times 100}{N \times V} $$

$ DI = $ disease index;
$ n_i = $ number of infected plants on the $ i $-th score;
vi = the i-th score value;
N = number of plants observed;
V = highest score

The disease scoring namely 0 = healthy plant, 1 = leaf slightly yellowing, but no necrosis on the stem, 2 = minor necrosis on the stem, 3 = moderate necrosis on the stems and plant slightly withering, 4 = severe necrosis and clearly visible plant withering, 5 = dead plant. The grouping of plant resistance is said to be resistant / resistant (R) if (DI<20), medium / moderate resistance (MR) (20<DI>50) and susceptible (S) (DI>50).

3. Result and discussion

3.1. Identification of *P. capsici*

The result of identifying *P. capsici* fungi isolated from symptomatic chili plants contain of macroscopic and microscopic characters as shown in Figure 1. The macroscopic character of the colonies is white, smooth like cotton, with uneven colony edges. Its microscopic character has branched and insulated hypha. The zoosporangium is shaped like a lemon (Figure 1C) and also looks like a pear/ellipse (Figure 1D). This is consistent with Watanabe [16] who stated that *P. capsici* sporangia are long ellipses, often triangular or irregular in shape, and the sporangiophores are easily damaged and separated. The zoospores are growing inside the sporangia.

![Figure 1](image)

*Figure 1*. Macroscopic and microscopic character of *P. capsici* fungi. Fungal colony (A), hypha (B), zoosporangium which contain zoospores (C1) dan sporangiofor (C2), and zoosporangium (D).

3.2. Inoculation

The results of *P. capsici* inoculation on chili seedlings obtained six varieties showing signs of infection, namely varieties 1, 2, 5, 6, 13, and 21. Meanwhile, twenty-four other varieties were not infected. The symptoms that appear are wilting and necrosis of the stems. The score for early symptoms of the infection appear on each plants are vary. The variation in symptom scores and the appearance time of the early symptoms can be seen in Table 1. In variety 13, the first signs of infection were scored 3 and 4. While in variety 2, the scores for the first symptom appeared were 3 and 5. The timing of the first symptoms of infection also varied. This is indicated by chili varieties 21 and 6. In variety 21, the first symptoms of infection appear at 7, 14, and 21 days after inoculation with variation of scores. Whereas in variety 6 the first symptoms of infection appeared on 21 and 28 days after inoculation.
**Table 1.** Variation in symptom scores and time of the first symptoms

| No | Infected Plant | Observation (day+) |
|----|----------------|--------------------|
|    |                | 7 | 14 | 21 | 28 | 35 |
| 1  | 1              | 0 | 4  | 5  | 5  | 5  |
| 2  | 1              | 0 | 0  | 0  | 3  | 3  |
|    | 2              | 0 | 0  | 0  | 5  | 5  |
|    | **total**      | 0 | 4  | 5  | 5  | 5  |
| 5  | 1              | 0 | 4  | 5  | 5  | 5  |
| 6  | 1              | 0 | 0  | 5  | 5  | 5  |
|    | 2              | 0 | 0  | 0  | 2  | 3  |
|    | **total**      | 0 | 4  | 5  | 5  | 5  |
| 13 | 1              | 0 | 3  | 4  | 5  | 5  |
|    | 2              | 0 | 4  | 5  | 5  | 5  |
|    | **total**      | 0 | 4  | 5  | 5  | 5  |
| 21 | 1              | 0 | 4  | 5  | 5  | 5  |
|    | 2              | 0 | 5  | 5  | 5  | 5  |
|    | 3              | 0 | 0  | 5  | 5  | 5  |
|    | **total**      | 0 | 5  | 5  | 5  | 5  |

Resistance response of thirty chili varieties based on its disease index can be seen in Table 2. Total of twenty-four chili varieties showed resistance to *P. capsici*, five varieties were classified as moderate resistant, and one variety was susceptible. The response of chili variety 21 was classified as susceptible, while the response of varieties lot number 1, 2, 5, 6, and 13 were classified as moderate resistance. The twenty-four varieties classified as resistant in the results of this study were not necessarily resistant to *P. capsici* isolates from other regions. Oelke and Bosland [16] stated that no chili cultivars were generally resistant to *P. capsici*. This may be due to the physiological races present in the *P. capsici*. Physiological races were grouped according to their reaction to a different set of cultivars. Kim and Hwang [17] stated that plants with different ages will show different level resistance.
The resistance response indicated by symptom scoring in this study differs within varieties and among varieties. As well as, the time when the first symptoms appeared. This variation happened is thought to be due to the environmental temperature which is less optimum for *P. capsici*. At the time of observation the greenhouse temperature was around 26-30ºC. Hord and Ristaino [10] stated that the optimum temperature for zoospores germination is 24ºC in vitro. Similarly with the results of the study by Granke and Hausbeck [18], this fungus can infect almost 100% of the fruit tested at 12ºC with a population of zoospores ≥ 5 × 10^3/mL. While in this study the zoospores population that being used was ≥ 5 × 10^6/mL. This suggested that temperature is a major factor in the success of *P. capsici* infection. It will be our interest to create more humid environmental conditions in the greenhouse during the inoculation process.

| No | Infected Plant | Observation day- | DI (%) | Category       |
|----|----------------|-----------------|--------|----------------|
|    |                | 7   | 14 | 21 | 28 | 35 |        |
| 1  | 1              | 0   | 4  | 5  | 5  | 5  | 20    | Moderate resistant |
| 2  | 1              | 0   | 0  | 0  | 0  | 3  | 3     | 12    |
|    | 2              | 0   | 0  | 0  | 0  | 5  | 5     | 20    |
|    | total          | 0   | 0  | 0  | 0  | 0  | 0     | 32    | Moderate resistant |
| 3  | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 4  | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 5  | 1              | 0   | 4  | 5  | 5  | 5  | 20    | Moderate resistant |
| 6  | 1              | 0   | 0  | 5  | 5  | 5  | 20    | Moderate resistant |
|    | 2              | 0   | 0  | 0  | 0  | 2  | 3     | 12    |
|    | total          | 0   | 0  | 0  | 0  | 0  | 0     | 32    | Moderate resistant |
| 7  | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 8  | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 9  | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 10 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 11 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 12 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 13 | 1              | 3   | 4  | 5  | 5  | 5  | 20    | Moderate resistant |
|    | 2              | 4   | 5  | 5  | 5  | 5  | 20    | Moderate resistant |
|    | total          | 40  |     |     |     |     |       |        |
| 14 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 15 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 16 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 17 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 18 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 19 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 20 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 21 | 1              | 4   | 5  | 5  | 5  | 5  | 20    | Moderate resistant |
|    | 2              | 0   | 5  | 5  | 5  | 5  | 20    | Moderate resistant |
|    | 3              | 0   | 0  | 5  | 5  | 5  | 20    | Moderate resistant |
|    | total          | 60  |     |     |     |     |       | Susceptible |
| 22 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 23 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 24 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 25 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 26 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 27 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 28 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 29 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |
| 30 | 0              | 0   | 0  | 0  | 0  | 0  | 0     | Resistant |

The disease index (score at 35 days after inoculation) and resistance level of thirty chili varieties
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