

Laboratory Assessment of IRR 400 Series Rubber Clones Resistance Against *Colletotrichum* Leaf Fall Disease

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ABSTRACT

Rubber (*Hevea brasiliensis*) is an important plantation commodity in Indonesia. However, its productivity has declined due to leaf fall disease caused by *Colletotrichum*. This study was conducted at the Physiology Laboratory of the Sei Putih Research Unit, North Sumatra, with the aim of identifying and comparing the resistance levels of IRR 400 series rubber clones against *Colletotrichum* sp. The study employed an experimental method using a non-factorial Completely Randomized Design (CRD) consisting of two factors, namely rubber clones and *Colletotrichum* sp. pathogen isolates, with three replications. The assessment was conducted by inoculating rubber leaves with the pathogen, followed by observations of the incubation period, morphological symptoms, infected leaf area, and disease intensity. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at the 5% significance level. The results indicated differences in resistance levels among the tested clones, comprising 12 IRR 400 series clones and four comparison clones, namely IRR 104, IRR 112, PB 260, and PB 330. Three clones, namely IRR 429, IRR 446, and PB 330, were classified as resistant, indicating their potential as superior rubber clones with resistance to *Colletotrichum* leaf fall disease.

Keywords: Rubber, *Colletotrichum*, IRR 400 Series, Clone Resistance

INTRODUCTION

The rapid development of the global economy has encouraged countries to actively participate in international trade. Export activities play an important role in promoting

economic growth and generating foreign exchange earnings. One of the sectors that contributes significantly to Indonesia's exports is the plantation sector, with rubber being one of its leading commodities. Indonesia is one of the world's largest natural rubber producers, ranking second after Thailand. In 2024, Indonesia's natural rubber production reached approximately 2.26 million tons, making a significant contribution to the global rubber supply. This condition demonstrates that Indonesia has considerable potential and opportunities in international trade, particularly in the rubber commodity sector. Improving rubber productivity and quality is essential to maintaining Indonesia's competitiveness in the global market (Fadhilah, 2022). The development of Indonesia's rubber industry has shown a declining trend in cultivated land area each year from 2016 to 2025, with an average annual decrease of 1.81%. Rubber production has also experienced a decline at a more significant rate of 3.89% per year, particularly since 2022. Over the past decade, most rubber plantations have been managed by smallholder plantations, accounting for approximately 88.6% of the total area, while state-owned plantations account for 5.2% and private plantations for 6.3%. According to data from the Directorate General of Estate Crops (Ditjenbun) in 2024, the national rubber plantation area decreased by 0.11% compared to 2023, declining from 3.153 million hectares to 3.149 million hectares. However, rubber production increased by 0.95%, from 2.240 million tons in 2023 to 2.262 million tons in 2024. Awareness of the impacts of climate change and global warming needs to be continuously strengthened. The effects of global warming and climate change include rising temperatures and drought conditions, which can inhibit plant growth, prolong the immature phase of plants, reduce crop yields, and increase the risk of drought in certain areas. Sudden weather changes can also lead to excessive rainfall, which may disrupt tapping activities, cause production losses, and increase the risk of disease attacks on plant leaves and roots (Fadhilah, 2022). Low rubber production is caused by various factors, one of which is the occurrence of plant diseases. Plant diseases can hinder growth and cause damage to various parts of rubber plants. Rubber diseases have resulted in economic losses amounting to billions of rupiah, not only due to production losses caused by plant damage but also because of the high costs required for disease control (Nikmah, 2022). Leaf fall diseases in rubber plantations are relatively difficult to control under field conditions. The selection of superior clones

is a more effective approach to disease management because such clones possess high levels of resistance to pathogenic organisms. There are four major types of leaf fall diseases affecting rubber plants, namely *Pestalotiopsis*, *Corynespora*, *Colletotrichum*, and *Oidium* (Kusdiana et al., 2023). *Colletotrichum* leaf fall disease is one of the major leaf diseases that reduce rubber plant productivity in various rubber-growing regions across Indonesia. This disease can decrease rubber productivity by 7–45%, depending on the severity of infection, ranging from mild to severe. The occurrence of *Colletotrichum* leaf fall disease is associated with irregular climatic patterns, particularly prolonged dry seasons followed by rainfall throughout the year, weakened plant conditions due to inadequate maintenance, and the cultivation of susceptible clones (Permana and Diyasti, 2022). Severe epidemics of *Colletotrichum* disease have been reported in Java, where the disease affected GT1 clones during 1973–1975, and in Sumatra in 1976. Furthermore, an outbreak occurred in West Kalimantan in 1989, affecting an area of approximately 24,000 hectares (Pasaribu et al., 2015).

MATERIALS AND METHODS

Place And Time

This study was conducted at the Physiology Laboratory of the Sei Putih Research Unit, located in Galang District, Deli Serdang Regency, North Sumatra Province, Indonesia, from June to August 2026.

Materials And Tools

The materials used in this study were as follows leaves of IRR 400 series rubber clones, *Colletotrichum* sp. fungal isolates, potato dextrose agar (PDA) medium, sterile distilled water, 70% alcohol, sterile cotton, filter paper, transparent plastic sheets, sterile tissue paper, sodium hypochlorite (NaOCl) solution, research labels, parafilm.

The equipment used in this study consisted of the following laminar airflow cabinet, autoclave, microscope, hemocytometer, hand sprayer, petri dishes, inoculating loop, forceps, scissors, dropper pipette, measuring cylinder, erlenmeyer flasks, analytical balance, documentation camera, incubator, mini hand sprayer, stationery and observation notebook.

Experimental Design

This study employed an experimental method using a non-factorial Completely Randomized Design (CRD), consisting of one treatment factor and three replications. The tested rubber clones consisted of 12 IRR 400 series clones and four reference clones. The treatment codes and corresponding clone names were as follows:

- K1 = IRR 425
- K2 = IRR 428
- K3 = IRR 429
- K4 = IRR 430
- K5 = IRR 431
- K6 = IRR 434
- K7 = IRR 436
- K8 = IRR 440
- K9 = IRR 446
- K10 = IRR 449
- K11 = IRR 451
- K12 = IRR 452
- K13 = IRR 104 (Reference Clones)
- K14 = IRR 112 (Reference Clones)
- K15 = PB 260 (Reference Clones)
- K16 = PB 330 (Reference Clones)

All tested clones were subjected to inoculation with *Colletotrichum* sp. under laboratory conditions to assess their resistance to the pathogen.

Research Stages

- Isolation of *Colletotrichum* sp.

  The pathogen was isolated from rubber leaves exhibiting symptoms of Colletotrichum leaf fall disease collected from an entres garden. Leaf tissue samples measuring approximately 1 × 1 cm were taken from the boundary between healthy and diseased areas. The samples were surface-sterilized using 70% alcohol for 30 seconds, followed by sodium hypochlorite (NaOCl) for one minute. Subsequently, the samples were rinsed three times with sterile distilled water and dried using sterile tissue paper. The sterilized tissue pieces were then transferred onto potato dextrose agar (PDA) medium and incubated at room temperature for 5–7 days. The fungal colonies that developed were subsequently subcultured to obtain pure cultures.
- Preparation of Spore Suspension

The spore suspension was prepared using a seven-day-old pure culture of Colletotrichum sp. Sterile distilled water was added to the fungal culture, and the colony surface was gently scraped to release the spores. The resulting suspension was filtered through sterile gauze to separate the spores from fungal mycelia and other debris. The spore concentration was determined using a haemocytometer and adjusted to approximately 10^6 spores/mL. The prepared suspension was then used for the inoculation of rubber leaves.

- Pathogen Inoculation

  Rubber leaves from each tested clone were placed in sterile trays or Petri dishes lined with moist sterile tissue paper to maintain humidity during incubation. The prepared Colletotrichum spore suspension was applied evenly to the leaf surfaces. Following inoculation, the leaves were covered with transparent plastic or maintained in a moist chamber to provide suitable conditions for infection and disease development. The inoculated leaves were incubated at room temperature for eight days.

- Disease Observation and Assessment

  Observations were conducted to evaluate the resistance responses of the rubber clones to Colletotrichum sp. Disease development was assessed based on the incubation period and disease intensity. The incubation period was determined by recording the time elapsed between pathogen inoculation and the appearance of the first visible symptoms, including brown spots, necrosis, and leaf discoloration. Disease intensity was observed on days 2, 4, 6, and 8 after inoculation. The assessment was based on a disease severity scale ranging from 0 to 4, with the following criteria:

Table 1. Disease Severity Scale

Score	Percentage of Affected Leaf Are
0	0%
1	1-25%
2	26-50%
3	51-75%
4	76-100%

Disease intensity was calculated using the following formula:

$$\text{IP} = \frac{\sum(\text{n} \times \text{v})}{\text{N} \times \text{Z}} \times 100\%$$

Where:

IP = Disease intensity (%)

n = Number of leaves in eache disease severity category

v = Disease severity score

N = Total number of leaves observed

Z = Maximum disease severity score

The resistance classification of each rubber clone was determined based on disease intensity according to Pawirosoemardjo (1999), using the following categories:

Table 2. Classification of Disease Intensity Caused by *Colletotrichum* sp.

Disease Intensity (%)	Resistence Classification
0-20	Very resistant
21-40	Resistant
41-60	Moderately resistant
61-80	Susceptible
81-100	Highly susceptible

- Data Analysis

  The data obtained from the observations were analyzed using analysis of variance (ANOVA) at a 5% significance level to determine differences in disease intensity among the tested rubber clones. When significant differences were found, the analysis was followed by Duncan's Multiple Range Test (DMRT) at the 5% significance level to identify differences among treatment means.

RESULTS AND DISSCUSSION

Development of Disease Intensity Following *Colletotrichum* Inoculation

The results showed that disease intensity caused by *Colletotrichum* sp. varied among the tested rubber clones during the observation period. In general, disease intensity

tended to increase over time, indicating differences in the responses of rubber clones to pathogen infection. The disease intensity recorded at 4, 6, and 8 days after inoculation (DAI) is presented in Table 3.

Table 3. Mean Disease Intensity of Rubber Clones Following Inoculation With *Colletotrichum* sp.

No.	Rubber Clone	4 DAI (%)	6 DAI (%)	8 DAI (%)
1	IRR 425	0,00a	88,89 ab	88,89 ab
2	IRR 428	5,56a	44,44 bc	50,00 bc
3	IRR 429	22,22a	38,89 c	38,89 c
4	IRR430	16,67a	61,11 abc	61,11 bc
5	IRR 431	5,56a	66,67 abc	83,33 abc
6	IRR434	5,56a	44,44 bc	50,00 bc
7	IRR 436	11,11a	66,67 abc	66,67 abc
8	IRR 440	0,00a	55,56 abc	61,11 bc
9	IRR 446	0,00a	27,78 c	33,33 c
10	IRR 449	5,56a	61,11 abc	83,33 abc
11	IRR 451	0,00a	66,67 abc	77,78 abc
12	IRR 452	5,56a	66,67 abc	88,89 ab
13	IRR 104	0,00a	33,33 c	55,56 bc
14	IRR 112	11,11a	94,44 a	100,00 ab
15	PB 260	11,11a	44,44 bc	55,56 bc
16	PB 330	5,56a	33,33 c	33,33 c

At 4 DAI, disease intensity ranged from 0.00% to 22.22%. Several clones, including IRR 425, IRR 440, IRR 446, IRR 451, and IRR 104, showed no disease symptoms based on their recorded mean values. However, IRR 429 exhibited the highest disease intensity at 22.22%. These results indicate that the initial responses of the tested clones to *Colletotrichum* sp. infection were variable.

At 6 DAI, disease intensity increased considerably, with mean values ranging from 27.78% to 94.44%. IRR 112 recorded the highest disease intensity (94.44%), followed by IRR 425 (88.89%). In contrast, IRR 446 showed the lowest intensity (27.78%), followed by IRR 104 (33.33%) and PB 330 (33.33%). These results indicate that the

initial absence or low occurrence of symptoms does not necessarily reflect sustained resistance, as disease development may become more apparent at later observation times.

At 8 DAI, disease intensity ranged from 33.33% to 100.00%. IRR 112 showed the highest disease intensity (100.00%), indicating a high level of susceptibility under the experimental conditions. Conversely, IRR 446 and PB 330 recorded the lowest mean intensity, both at 33.33%, followed by IRR 429 at 38.89%. These three clones therefore showed relatively lower disease development than the other tested clones.

The increase in disease intensity over time indicates that evaluating clone responses at multiple observation times is important. Observations conducted only during the early stages after inoculation may not adequately represent the subsequent development of disease. Therefore, the final observation provides additional information for identifying clones with relatively low disease intensity.

Identification of Clones with Relatively Low and High Disease Intensity

Based on the final observation at 8 DAI, IRR 446, PB 330, and IRR 429 showed the lowest disease intensity, with mean values of 33.33%, 33.33%, and 38.89%, respectively. These results indicate that the three clones had relatively better responses to *Colletotrichum* sp. infection under the laboratory conditions used in this study.

In contrast, IRR 112 showed the highest disease intensity, reaching 100.00%, followed by IRR 425 and IRR 452, both with mean values of 88.89%. The high disease intensity in these clones suggests that they were more susceptible to infection during the observation period.

The relatively low disease intensity in IRR 429 and IRR 446 is consistent with findings reported by Pasaribu et al. (2015), who evaluated the resistance of IRR 400 series rubber clones against *Colletotrichum*. Their study also reported variation in resistance among rubber clones, including several IRR 400 series clones with relatively high resistance responses.

The identification of clones with lower disease intensity may provide useful information for rubber clone selection and disease management. However, resistance

classification should consider the established disease severity criteria and further validation using different pathogen isolates and field conditions.

Implications for Rubber Clone Selection

The variation in disease intensity among the tested clones provides useful information for the evaluation and selection of rubber planting materials. Clones that consistently exhibit lower disease intensity may be considered promising candidates for further resistance testing. In this study, IRR 446 and IRR 429 showed relatively low disease intensity among the IRR 400 series, while PB 330 showed a similarly favorable response among the reference clones.

These findings are relevant to rubber cultivation because the use of planting materials with better disease resistance can contribute to integrated disease management. However, clone selection should not rely solely on laboratory disease intensity. Agronomic performance, latex productivity, adaptability, and resistance to other important diseases should also be considered.

The results also highlight the importance of further research using multiple *Colletotrichum* isolates. Differences in pathogen isolates may influence disease development and the expression of resistance in rubber clones. Therefore, greenhouse and field evaluations are needed to determine whether the relatively favorable responses of IRR 446 and IRR 429 can be maintained under different environmental and pathogen conditions.

CONCLUSION

Based on the laboratory evaluation of the resistance of IRR 400 series rubber clones to *Colletotrichum* leaf fall disease, it can be concluded that the tested clones exhibited different responses to *Colletotrichum* infection. The analysis of variance showed that clone treatments had a significant effect on disease intensity at all observation times. These differences were particularly evident on days 6 and 8 after inoculation, indicating variations in clone responses to disease development. At the final observation (8 days after inoculation), three clones were identified as resistant, namely IRR 429, IRR 446, and PB 330.

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