Floating Leaf Disc Assay In-Vitro: Evaluation of Satoimo Taro Cultivar (Colocasia esculenta) Resistant to Phytophthora colocasiae Causing Leaf Blight Disease

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**Floating Leaf Disc Assay In-Vitro: Evaluation of Satoimo Taro (Colocasia esculenta) Resistant to Phytophthora colocasiae Causing leaf blight Disease**

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**Abstract:** The study aims to examine Saitomo cultivar resistance to *phytophthora colocasiae* isolate causing *leaf blight* disease following *floating leaf disc assay* method in vitro. This research was conducted in Sentoso Hamlet, Lekopancing Village, Kec. Tanralili, Maros Regency. and at the Plant Disease Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Hasanuddin University, Makassar. The research was carried out September 2019 to May 2020. Leaf samples were obtained from Satoimo cultivar of taro. Pathogen isolation was subcultured onto juice V-8 medium before putative *Phytophthora* *colocasiae* isolate was tested into 2 cm square of leaf following *floating leaf disc assay* technique. Symptomatic development of necrotic lesion was the highest in the old leaf as well as disease incidence while the lowest was in mature leaf. Pathogen isolation obtained from a wild leaf taro with necrotic lesion had similarity character with *Phytophthora colocasiae* species.

**Keywords:** *Phytophthora colocasiae*, leaf blight disease, Satoimo taro, floating leaf disc assay

1. **Introduction**
   Taro is a potential crop as staple food in the tropics particularly Indonesia to substitute rice, sago, corn or cassava and to strengthen food security. Taro generally grows in opened- forest areas or under the shade. Taro riches at a source of carbohydrates and with tuber, taro has bioactive ingredients for health due mainly to phenolic substances [1]. In the future the need for alternative carbohydrate is vital and taro can play a role. Demand of tuber export is prmosingly as Japan is potential market with 100 tonnes demand per month. In the farm scale, tuber production is still low with 20 tonnes per ha [2] and the increase of taro production to supply food security and international market is necessary. Unfortunately, similar other crops infected by Phytophthora species, the disease always becomes main limiting part in crop development [3-5].
   
   Taro Leaf blight caused by *Phytophthora colocasiae* is the most obvious damaging disease threatening global taro industry [6-8]. The pathogen is known as a narrow association with the pathogen species, *P. infestans*, posing tragically famine in Irlandia due to calamitous destruction of potato crops [8]. The pathogen infects all important parts of plant and every stage of taro development. The infection causes leaf lesion and if infection continues to develop, severe host occurs particularly vulnerable cultivar that impacts to undeveloped tuber. The agent of disease can also cause corm rot [6]. Rapid spread of disease and pathogen in environment is mainly caused by a typical soil borne pathogen due to moveable spores
and a thick-walled spore (clamidospore) that is generated to survive in extreme weather and environment. The pathogen has a wider-host range \([6,9]\) and can survive in the soil for long term \([10]\).

Furthermore, disease spread can also be helped in many ways such as equipment contamination, disease carrier by insect, humidity, temperature and infected tissue connection. Taro corms are susceptible to infection by *Phytophthora colocasiae* \([6]\).

To cope with pathogen spread and yield loss due to leaf blight disease in developing taro industry in the future, seeking resistant cultivar is very important and one of components is by assay in vitro \([11]\). Hence, this study was undertaken to examine resistance of taro Satoimo cultivar, potential high yield, to *Phytophthora colocasiae* isolate in vitro that may contribute to profile resistant taro cultivar in the scale up stage.

2. **Material and Methods**

Sample collection with leaf blight and healthy leaf signs was undertaken in Sentoso Hamlet, Lekopancing Village, Kec. Tanralili, Maros Regency. For putative *P. colocasiae*, native isolate was obtained from wild taro community. Testing leaves of interest was undertaken at the Plant Disease Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Hasanuddin University, Makassar. The study took place from September 2019 to May 2020.

2.1 **Subculture preparation.** Subculture juice V-8 medium was used to maintain putative *P. colocasiae* following Drenth \([6]\) method. The medium consisted of 15 grams agar, CaCO₃, 2 grams, 200 mL of V-8 juice and 800 mL of distilled water for the manufacture of 1 liter, then autoclaved at 121°C for 2 hours, then added Chlorompenicol ½ capsule.

2.2 **Symptomatic leaf isolation.** Leaf of interest was sterilized with 70% Ethol. The infected leaf was cut small pieces (2 cm square) following ½ lesion and ½ healthy tissue. All activity was undertaken in the laminar airflow. The tissue (lesion and health) was submerged into 70% Ethol. Sequence events of surface sterilization consisted of leaf submersion with 1 min 70% Ethol, 1 min 2% NaOCl and 30 sec sterilized water before the leaf tissue was dried with sterile filter paper. Dried tissue was transferred into subculture juice V-8 medium. 4 tissues were placed parallelly in a petridish.

2.3 **Putative Phytophthora colocasiae isolation and detection.** Putative isolate was collected from infected wild leaf taro with leaf blight sign. It was then characterized morphologically including mycelium, sporangia shape, hyphal swelling, papillation of sporangia etc. Purifying putative isolate was undertaken in the juice V-8 medium \([6]\). Putative isolate growing onto juice V-8 medium was cut with cork borer and transferred into a new subculture juice V-8 medium. Whole isolation activity was conducted with sterilized place and tools.

2.4 **Testing Satoimo cultivar using floating leaf disc assay method.** Testing pathogenicity with floating leaf disc assay was previously demonstrated by Nath \([11]\). The procedure is quite simple but accurates to reach at Koch’s postulate purpose. The procedure consisted of that healthy leaf was cut into a 2 cm square and subsequently inoculated putative *P. colocasiae* isolate laid into leaf central. A 2 cm square leaf with isolate was floated in the sterilized water surface on the petri dish. The leaf of interest was let to float under humid and dark condition and every day symptomatic lesion development on leaf surface was observed.

2.5 **Lesion development and disease incidence.** Lesion development was counted with measuring the length of necrosis on leaf trial in mm. For disease incidence, it was statistically analyzed with following: number of infected leaves divided into total leaves observed times 100 percent.

3. **Results and discussion**

3.1 **Pathogen isolation and identification**

Histologically, putative *Phytophthora colocasiae* obtained from symptomatic leaf blight on wild taro had similarity with the origin of the causal disease agent of leaf blight disease on taro \([6]\). For more detail, the description of the pathogen was shown below (figure 1).
Figure 1. (A) leaf blight symptom on wild leaf taro with dark spots and yellowing in marginal leaf. Infected leaf tissue was inserted to isolate onto Juice V-8 medium (red square); B) mycelium inserted and transferred onto glass microscope (red square) shown to have cotton-like mycelium appearance on the Juice V-8 medium; C) sporangia emerged on subculture; D) dark thick-walled hyphae (black arrow) and ‘ovoid’ sporangia shape (red arrow) with semi-papillate and short-pedicle sporangia (white arrow) obtained from morphological identification of putative Phytophthora colocasiae; E) Semi-papillate shape of sporangia. Tremendous spores were generated in a sporangium.

Sporangium shape, papillation of the sporangium and hyphal swelling are the important clues to characterize Phytophthora genus particularly P. colocasiae species [6]. In the study, the observation found that sporangia obtained from putative isolate shapes like ovoid and is semi-papillate as well as short-pedicle sporangia (figure 1 D-E). Typical hyphal swelling is absent on the isolate of interest. Whole characters shown in the study to have similarity of P. colocasiae causing leaf blight disease explained in key determination of Phytophthora species. Overall, it evidences that leaf blight lesion on taro is only caused by a single causal-disease agent, Phytophthora colocasiae [6,9].

3.2 Development of symptomatic taro leaf blight
In general, statistical analysis shown that, a significant lesion development occurred in old leaf of Satoimo cultivar after a week observation and continued to rise until the end. In contrast to old leaf on this test, the lesion development on immature and mature leaf was likely to develop insignificantly from the beginning to the end of observation compared to control. In this control, no putative P. colocasiae was infected to immature, mature and old leaves.
**Figure 2.** Necrotic lesion development in different leaf trial. Control consisted of immature, mature and old with putative *P. colocasiae*

**Figure 3.** Demonstration of floating leaf disc assay of Satoimo cultivar. A) 4 days after inoculation. The lesion absent. B) 12 days after inoculation. A significant development of leaf blight necrotic lesion. It indicates that lesion developed from the epicentral putative isolate laid in the central of leaf showing with leaf coloration.

**Figure 4.** Disease incidence (%) signed with necrotic lesion on Satoimo cultivar leaves by the time. No disease development occurred in Control.
Figure 5. Average of disease incidence (%) of Satoimo cultivar leaves in entire observation (day) in different leaf development

4. Discussion
Disease incidence and development of leaf blight lesion on taro Satoimo cultivar significantly occurred in old leaf. Disease incidence in old leaf trial progressively increased by the time while disease incidence in immature and mature leaves was stagnant (figure 4). The necrotic lesion in the leaf floating disc assay and disease development shown that necrosis rapidly developed after 10 days after inoculation (figure 2-3). Overall, disease incidence significantly occurred in old leaf (figure 5). One of main causes behind rapid development of necrotic lesion on old leaf of Saotomi cultivar is mostly caused by undermining host-immune by the time and after virulence putative isolate tested is still available. Old leaf of taro experiences more significant impact of the lesion. rapid lesion development commenced at 8 days until a complete necrotis tissue in the end (figure 2; figure 3B). In constrast, mature leaf is more resistant to putative *P. colocasiae* isolate than other trials. This indicates that once the leaf develops in a month, old leaf immunity tends to drop and symptomatic necrotic lesion rapidly occurs. Another is leaf canopying to underpin the disease development. Old leaf site becomes shade once the rise of new leaf is generated to cause humidty as well as adjasent rhizosphere where pathogen contactes [10].

5. Conclusion
Overall, old leaf of Satoimo cultivar was much more suscaptable than immature and mature leaves. Disease development of leaf blight necrotic lesion and disease incidence were the greatest again in the old leaf trial. Mature leaf was the lowest. The causal disease-agent of leaf blight necrotic lesion on the infected leaf obtained from a wild taro had a similar character with *Phytophthora colocasiae* species.

6. Reference


