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# MORPHOLOGICAL AND MOLECULAR VARIATION OF *PHYTOPHTHORA COLOCASIAE* IN 'SATOIMO' TARO (*COLOCASIA ESCULENTA* VAR ANTIQUORUM) IN INDONESIA

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**A B S T R A C T**

Taro leaf blight is a harmful disease caused by *Phytophthora colocasiae* Raciborski and has become the primary factor in damaging 'satoimo' taro. A survey conducted in the agriculture fields of satoimo taro (*Colocasia esculenta* var antiquorum) in Indonesia indicated its severity disease scale between 25-50%. Morphological and molecular techniques were used to characterize the population of *Phytophthora colocasiae* obtained from different locations in Makassar, Maros and Gowa districts. Based on colony growth patterns, the isolates exhibited stellate and petaloid. The mean sporangial length of isolates of *P. colocasiae* range from 37.06 and 53.80 µm and mean width range from 22.27 and 26.90 µm. *P. colocasiae* are highly dynamic in nature and a considerable degree of diversity exists among them. This provides an important basis for developing suitable control against leaf blight of taro caused by *P. colocasiae* based on good integrated approach. Based on the phylogenetic analysis, *P. colocasiae* isolates (PCEMK01 and PCEMR01) was exhibited in the same cluster with *P. colocasiae* strain 3 reported from India with the bootstrap of 96% at 500 replicates in MEGA 5.

**Keywords**: Taro leaf blight, Satoimo Taro, *Phytophthora colocasiae*.

## INTRODUCTION

Taro leaf blight is a devastating disease of 'satoimo' taro (*Colocasia esculenta* var *antiquorum*), caused by *Phytophthora colocasiae* Raciborski ( Mbong *et al.,* 2013). Severe infection can defoliate all leaves and petioles, ending with death and decay, that caused failure in the production of corm (Bandyopadhyay & Sharma, 2011; Omane *et al*., 2012). Crop failures in some areas of Oceania reached 25-50% and even more massive than 90% in South Asia and West and Middle Africa (Misra *et al.,* 2008: Bandyopadhyay and Sharma, 2011). The infection of taro leaf blight has reduced the production rate of taro (C*olocasia esculenta*) by 30-40% in American Samoa. Due to these heavy losses, quantity and the quality of corms is reduced and hinder the commercialization of the taro corm product in Indonesia and taro growing other countries also (Bandyopadhyay and Sharma, 2011; Mbong *et al.*, 2013).

*P. colocasiae* are highly dynamic in nature and a considerable degree of diversity exists among them (Adomako *et al.,* 2018; Nath *et al.,* 2014; Padmaja *et al.,* 2017).

Therefore, knowledge related to the morphological and molecular characteristics of *P. colocasiae* from Indonesia is necessary to find out the right strategy to control the. This is the first study in Indonesia investigating taro leaf blight disease to the best of our knowledge.

## MATERIALS AND METHODS

**Survey disease severity in Taro Field:** Taro Leaf Blight infected leaves were collected from different Taro growing areas of South Sulawesi, Indonesia. (Makassar, Maros dan Gowa districts).

The 'satoimo' taro plants were categorized by 0-5 scale disease rating scale for percent leaf blight (Padmaja, 2013; Padmaja *et al.*, 2017)

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The percent disease index (PDI) was calculated using the formulas:

$$PDI = \frac{\sum Sum\ \ of\ \ all\ numerical\ ratings}{\text{Total number of leaflets observed x maximum rating}}\ x\ 100\%$$

Table 1. Disease severity scale of taro

| Disease Scale | Description of symptom | Percent Disease Index |
|---|---|---|
| 0 | Leaves free from infection | 0 |
| 1 | Former of infection less than 1% | <1% |
| 2 | Slight of infection, lesion visible up to 5% | 1.01-5% |
| 3 | Moderate infection, lesion coalesce at 5-25% | 5.01-25% |
| 4 | Heavy infection, lesion visible on 26-50% | 25.01-50% |
| 5 | Severe infection, lesion more than 50% | >50% |

Table 1. Disease severity scale

| Scale | Inference | Disease severity (%) |
|---|---|---|
| 0 | No infection | 0 |
| 1 | Low infection | <1 |
| 2 | Low infection | 1.01-5 |
| 3 | Moderate infection | 5.01-25 |
| 4 | High infection | 25.01-50 |
| 5 | Very high infection | >50 |

**Sampling and the isolation of the pathogen:** TLB infected leaves were collected from the sample farms from the three districts in South Sulawesi, Indonesia. Then the samples were washed in running water. The surface sterilization with 70% ethanol for 1 minute followed by dipping into sterilized water was done. After that, the infected leaves were cut into smaller pieces (1 $cm^2$) and dried using Whatman filter paper. Then cutted pieces were placed into V8 juice agar (100 ml V8 juice, 900 ml water, 20 g agar, 2 g $CaCO_3$, 0.1 mL nystatin & 0.1 g ampicillin). The plates were kept in an incubator for seven days, and observed for macroscopic colonies' growth patterns (Padmaja *et al.,* 2017).

**Morphological characterization:** Isolates that were being grown in V8 media, after seven days, their morphological characteristics were observed. Macroscopic features were observed as color, texture, and growth pattern of the colonies (Tariq *et al.,* 2015). Microscopic observation (40X magnification) was also carried out along with the sizes and shapes, the forms of the fungal sporangium, sporangiophore, and the appearance *P. colocasiae* papilla.

**Molecular Identification :** *P.colocasiae* were grown in V8 medium (komposisi). DNA was extracted from 5 to 7 days old cultures according to Misra *et al,*(2008). The isolated DNA was subjected to Internal Transcribed Spacer (ITS) 1 and 4. ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3' (White *et al.,* 1990). PCR was performed in a 25 µl reaction mixture containing: 10 ng DNA template, deoxynucleotide triphosphate 100 µM, 20 ng primer, 1.5 mM $MgCl_2$, 1x Taq Buffer (10mM Tris HCl pH 9, 50 mM KCl, 0.01% gelatin), The PCR Thermocycler conditions were 2 min at 94°C for denaturation, 30 cycles of 30 s at 94°C, 30 s at 50°C for annealing and 1 min at 72°C, followed by 8 min at 72°C for final extension. Amplifications were performed in duplicate for each sample, and the presence of the predicted PCR products was confirmed by 2% agarose gel electrophoresis and photographs were scanned through a Gel Doc System (Biorad Country). The BLASTn was performed for the sequenced nucleotide sequence and compared with the sequence data of *P. colocasiae* available in the GenBank using the BioEdit sequence Alignment ClustalW Program (Hall, 1999).

The output of the sequencing was analyzed using MEGA package version 5 (Tamura *et al*., 2011). To find out the compatibility of sequences that were also amplified with ITS primers (ITS1 / ITS4). Nucleotide accessed sequences from the GenBank. The access numbers were JN624814.1 (India), KY432681.1 (India), GU111605.1 (Taiwan), JN661147.1 (Hawaii), AB367386 (Japan), MH401210.1 (Sri Lanka), JN662439.1 (Ghana), AJ516027.1 (Indonesia), AJ516029.1 (Philippine) while for the isolate outside the group, we used JF799874.1 as *Phytophthora citrophthora* isolate *36087*. The analysis of the correlation among isolates employed the neighbor-joining method by Saitou & Nei (1987) The phylogeny was analysed using bootstrap with 500 repetitions

## RESULTS

**Disease Severity :** The results showed that disease severity of Tar Leaf Blight in South Sulawesi was 25-50%. The highest disease severity (50.31%) was observed in Gowa district. The disease severity observed in Makassar (25.02%) and Maros (24.98%) were

statistically the same.

The leaves formed many brown lesions that produced orange exudates. White sporulation was evident on the surface of the leaf when rainy conditions (Omane *et al.*, 2012). Petioles infected by pathogen showed spots, defoliation and become decayed (Figure1).

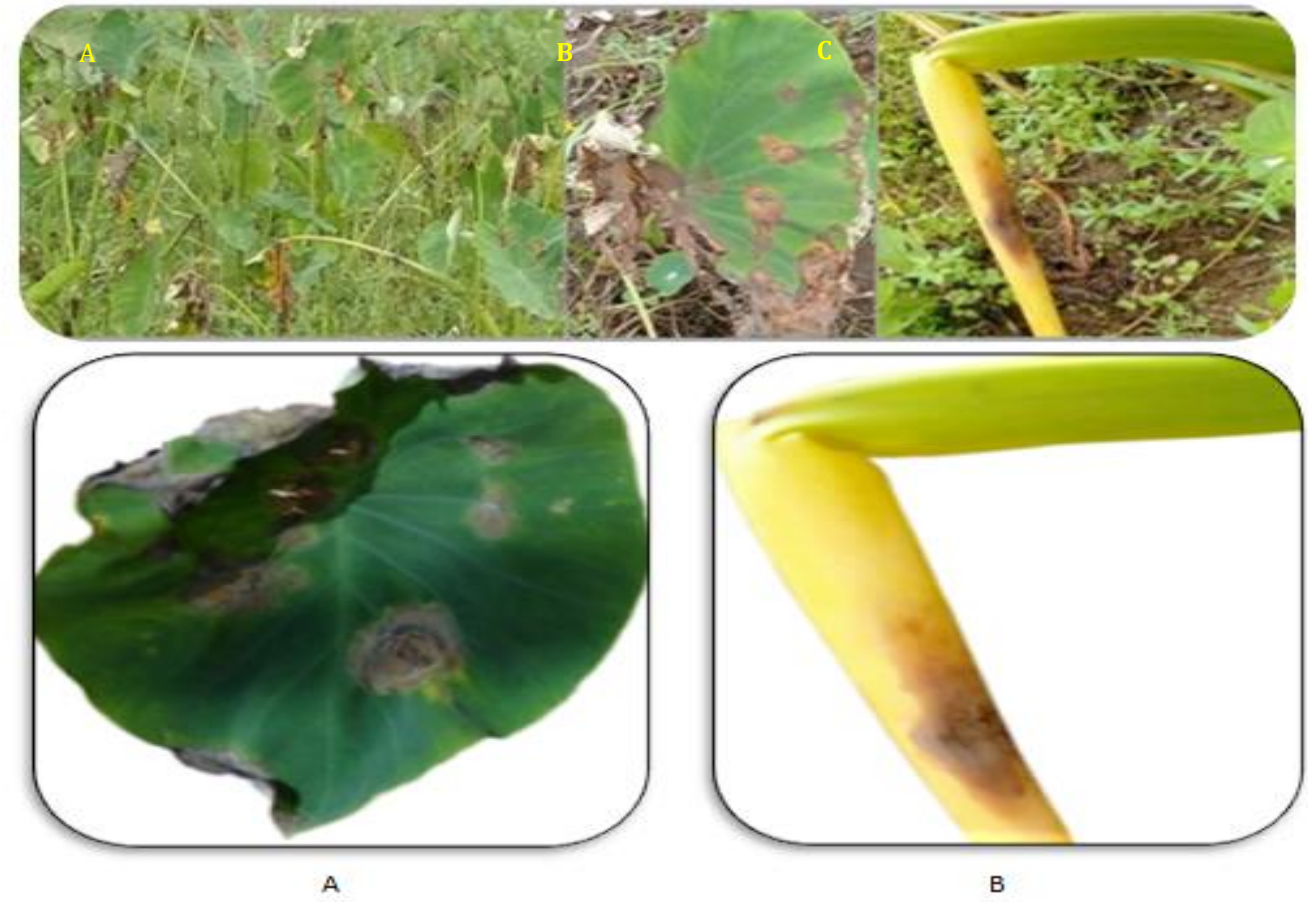


Figure 1.Symptoms of *Phytophthora* of taro plants from surveyed fields, A) leaf, B) petiole.

**Morphological Characterization of *P. colocasiae*:** Colony growth patterns were described from 3 d old culture grown at 20°C in the dark on V8 and potato-dextrose agar (PDA). Isolates tested from Makassar district (PCEMK01, PCEMK02) and from Maros district (PCEMR01, PCEMR2) exhibited two growth patterns in cultures: stellate and petaloid on V8. While cottony colonies are produced on both media. In the V8 medium, the mycelium, which was formed was thin and disappear. While in the PDA medium, the mycelium was dense and appeared to the surface. (Figure 2).

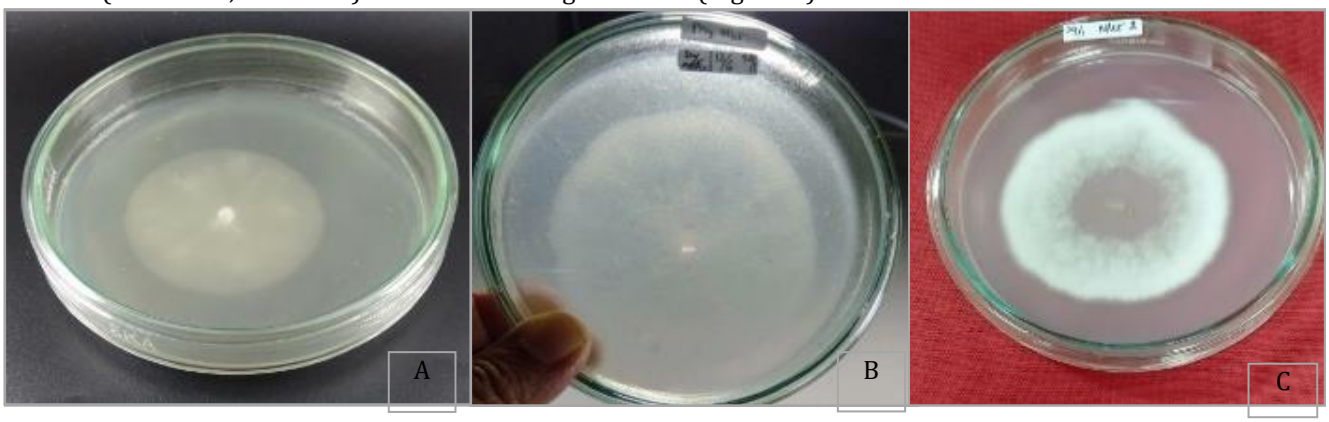


Figure 2. The growth pattern of *P. colocasiae* in the V8 agar (A,B) and PDA medium (C)

**Sporangium Morphology:** Sporangium characters was observed for all the isolates using microscope at magnification of 40x. Isolates produced semi papillate and papillate. The forms of sporangium included ovoid, ellipsoid, fusiform, tapered base with sporangial length from 37.06 and 53.80 µm and width range from 22.27 and 26.90 µm . Sporangiophore is simple sympodial and the hyphal swellings were absent (Figure 3).


A B C

Figure 3. A. The observation of hyphae *P. colocasiae*, *B. Sporangium* of *P. colocasiae*, C. Type of sporangiophore (40X magnification)

**Molecular Identification:** The primers ITS 1 and ITS 4 amplified regions of 850 bp for both PCEMK01 and PCEMR01 isolates. (Figure 4). Analysis of the sequences showed highest nucleotide identity (96%) to *P. colocasiae* strain PC 3 (JN624814.1) from India.

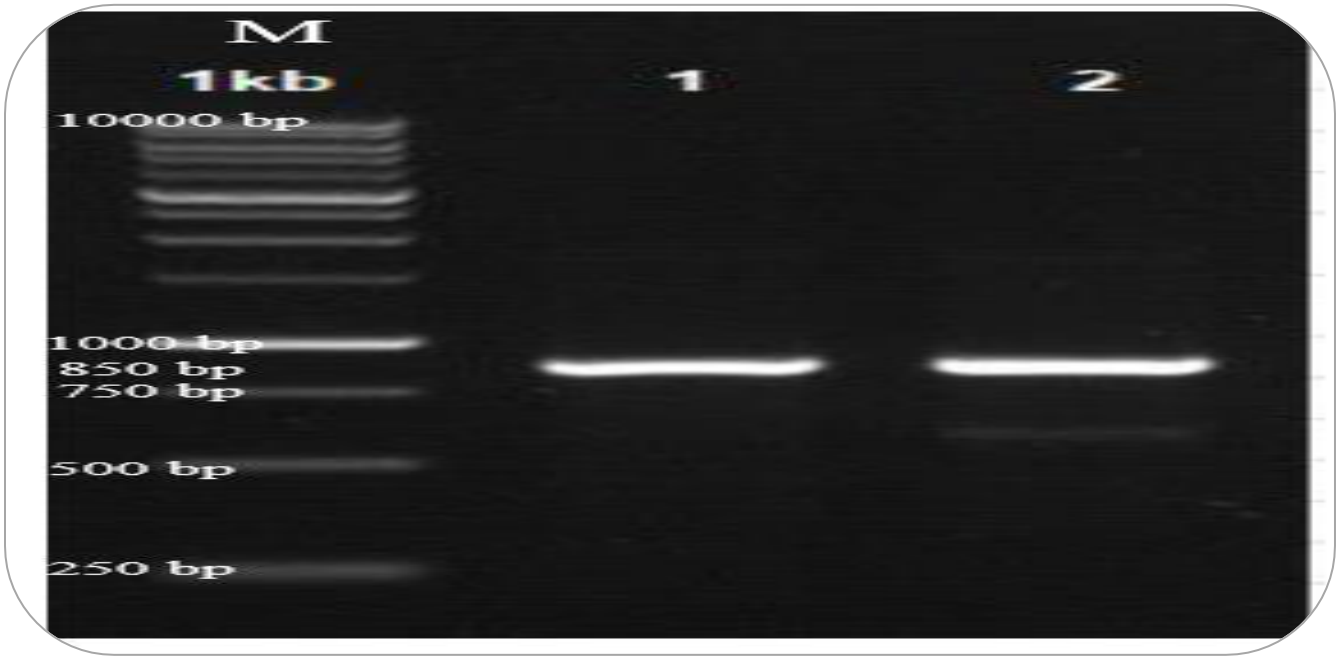


Figure 4. PCR amplified product on 2% gel (M: DNA ladder 1 kb, lane 1: isolate PCEMK01, lane 2: isolate PCEMR01)

The results obtained that the isolate PCEMK01 and PCEMR01 were *Phytophthora colocasiae* and have the same sequence of nucleotide bases with *P. colocasiae* isolates available in Gen Bank was 95.39% with *P. colocasiae* PC3 strain (Figure 5). The dendrogram results showed that isolates have a kinship relation of 96% with *P. colocasiae* strain 3 (JN624814.1) from India and very different from *P. citrophora* isolates which are isolates outside the *P. colocasiae* group.

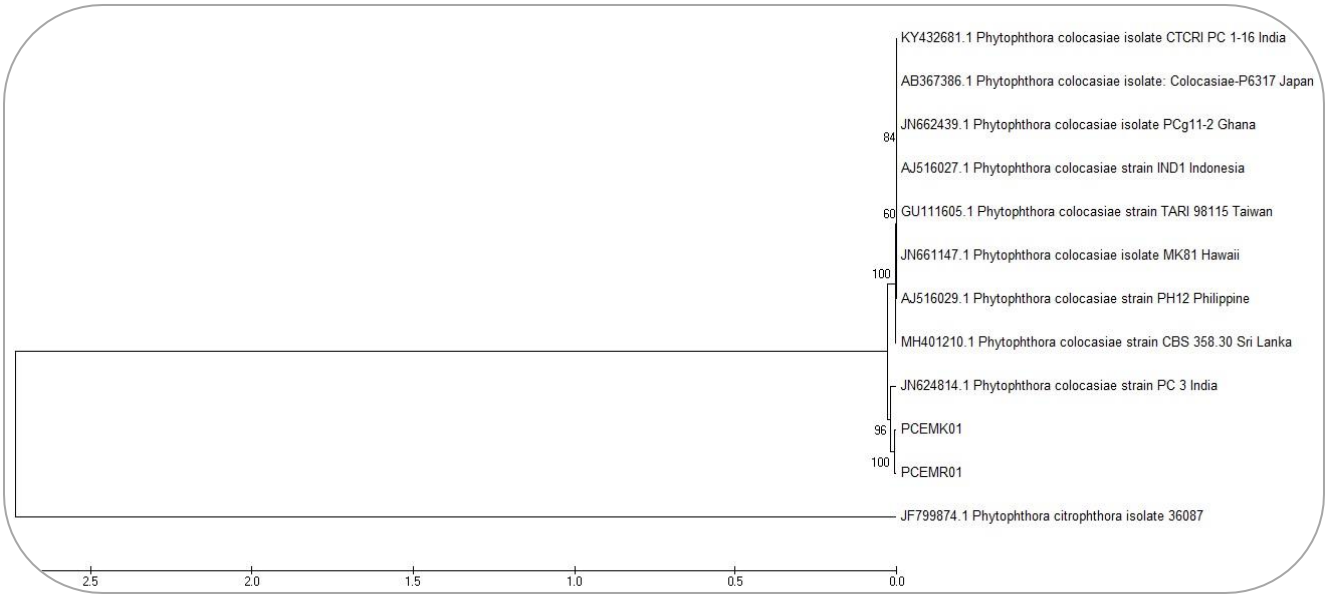


Figure 5. Dendrogram *P. colocasiae* isolate (PCEMK01 and PCEMR01) and some isolates published sequences from the Gen Bank with *P.citrophthora* was used as outgroup (bootstrap 500 replicates)

**DISCUSSION**

Isolates have various growth patterns at the culture media. In the V8 medium, the types were petaloid and stellate, while in the PDA medium, they isolate colonies were cottony. This conformed studies conducted by Misra (2011) and Padmaja *et al.* (2017), which found some growth patterns in the selective Phytophthora medium, which were cottony, rosaceous, stellate, and petaloid. While Nath *et al*. (2014) found two morphotypes: stellate and cottony. The *P. colocasiae* isolate shows various patterns of colony growth depended on the culture media, which was being used by Mbong *et al*. (2015); Nath *et al*. (2014); Tsopmbeng (2012) and in general, the growth of *Phytophthora* would be slow when it is grown in the culture media.

The microscopic observation showed that there was biological variation in the population of the pathogen, which can cause leaf blight disease. From the observation conducted on the mycelium, we identified that hypha was aseptate, hyaline, and did not form swollen hyphae (Drenth and Sendall, 2001). This was in line with Adomako *et al.* (2018); Bandyopadhyay (2011); Omane *et al.* (2012) discovering hyphae without septa and hyaline. The length sizes of the sporangium ranged between 37.06 and 53.80 µm. This is longer than then ones found Adomako *et al.* (2018), which were 21.2 -24.3 µm. however, they were almost similar to the ones discovered by (Omane *et al.,* 2012) between 25 and 55 µm, 38-60 µm (Misra *et al.,* 2011) and 30-60 µm (Bandyopadhyay and Sharma, 2011).

While the width sizes were between 22.27 and 26.90 µm. The width was almost the same with Omane *et al.* (2012), which 15-30 µm, Bandyopadhyay and Sharma (2011) 17-28 µm, but was wider than Adomako *et al.* (2018), which were between 14.9-17.2 µm. The forms of sporangium included ovoid, ellipsoid, fusiform, and tapered base. It is more various than Bandyopadhyay and Sharma (2011), which only detected ovoid. While Misra *et al.* (2011) and George *et al.* (2018) found two forms of the sporangium, which were ellipsoid and ovoid. Adomako *et al.* (2018) and Padmaja *et al.* (2017) identified ovoid and globose. Tsopmbeng *et al.* (2012) discovered three forms of the sporangium, which were ovoid, fusiform, and ellipsoid. While the tapered base was also found in *P. colocasiae* (Drenth and Sendall, 2001). Papilla sporangium had two forms, including semi papillate and papillate. A similar type of papilla was found by Adomako *et al.* (2018). According to Omane *et al.* (2012), and Bandyopadhyay and Sharma (2011), only found papillate papilla while George *et al.* (2018); Misra *et al.* (2011); Tsopmbeng *et al.* (2012) revealed semi pappilate papilla. Sporangiophore only had a simple sympodial, while Adomako *et al.* (2018) discovered sympodial and monopodial sporangiophore. Based on the grouping by Waterhouse (1963), *Phytophthora collocasiae* is included in the Phytophthora group IV.

The dendrogram shows that PCEMK01 and PCEMR01 isolated have a kinship relation of 96%, which is closer

to *P.colocasiae* strain 3 from India (JN624814.1), in bootstrap with 500 replication. Although *P. colocasiae* IND 1 (AJ516027.1) is also from Indonesia, the dendrogram showed if that isolate has a different cluster with isolates samples (PCEMK01 and PCEMR01) which are also from Indonesia. It is similar to the isolates *P.colocasiae* strain 3 (JN624814.1), and *P. colocasiae* isolate CTCRI PC 1-16 (KY432681.1), both of them were from India, but the phylogenetics showed that both isolates had different clusters although were from the same country. The dendrogram also indicated that *P. colocasiae* isolates were divided into some cluster groups. This proved that there is genetical variation among *P. colocasiae* isolates, although they were the same country. The isolate grouping does not indicate any correlation between the genetic factor and geographical distance (Nath *et al.,* 2012; Nath *et al.,* 2014). A similar study conducted by Lebot *et al.* (2003); Mishra *et al.* (2010) based on molecular like RAPD, isozyme, and some studies found a weak correlation between geographical location and isolate samples. Some factors are causing genetic variations. One of them is the gene mutation. The mutation is considered as the main contributor to gene variation (Goodwin, 1997). An observation conducted by Nath *et al.* (2014) showed that the *P.colocasiae* frequently mutate within a country, allowing the creation of new virulence in pathogenic strain.

Therefore, the effort to develop the efficient and continuous management strategies of leaf blight disease needs understanding on the phenotypic and genotypic variations of *P. colocasiae* pathogen through the characterization and the study of morphology and genetics of plants pathogen

## CONCLUSIONS

The results obtained that the isolate PCEMK01 and PCEMR01 were *Phytophthora colocasiae* and have the same sequence of nucleotide bases with *P. colocasiae* isolates available in Gen Bank was 95.39% with *P. colocasiae* PC3 strain. . The length of sporangium between 37.06 and 53.80 µm and the width of between 22.27 and 26.90 µm. Isolates tested exhibited three growth patterns in cultures: stellate, petaloid and cottony.

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| | | |
|---|---|---|
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| Tutik Kuswinanti | : | Designed and performed experiments, molecular identification |
| Ade Rosmana | : | Analyzed data and cowriter of paper, molecular identification |
| Andi Nasruddin | : | Analyzed data and cowriter of paper, molecular identification |