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Neck Rot Disease Caused by *Athelia rolfsii*, the Main Disease in Seedling of Elephant Foot Yam in Bali

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Abstract

Several tuber crops have been used as sources of carbohydrates based food. One of them is Elephant foot yams (*Amorphophallus muelleri* Blume). In addition to carbohydrate, this plant is also contain, fats, protein, minerals, vitamins and fiber. Cultivation of this plant usually started with seedling preparation made of tuber, bulbil or seed. However, during seedling preparation, several diseases appear and resulted in serious damage to the seedling. This study was done to identify the main diseases appeared during seedling preparation of elephant foot yam. The diseases survey was done in three centers of elephant foot yam seedling preparation namely Asah Duren Village, Bading Kayu Village, Jembrana Regency, Belalang Village, Tabanan Regency Bali. In each location, three seedling beds of 500 seedlings were observed to determine disease incidence based on disease symptom. Sample of diseased seedlings were taken and brought to the laboratory for identification. Analysis of 18S rRNA gene was done to identify the causal fungus. Results of this study showed that the main diseases of elephant foot yam seedlings in Bali were neck rot and leaf blight diseases, with disease incidences were 18.7% and 3.1% for neck rot and leaf blight disease respectively. Based on molecular identification, the isolate Pr1 as the cause of the neck rot disease is *Athelia rolfsii.* This isolate showed 99.12% similarity with 18S rRNA genes of several fungal strains deposited in GenBank. This study suggested that the main disease in seedling of elephant foot yam in Bali is neck rot disease caused by *Athelia rolfsii* Pr1.

Keywords: main diseases, elephant foot yams, seedling preparation

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1. Introduction

Elephant foot yam (*Amorphophallus muelleri* Blume) has long been used as food and as industrial raw materials (Saleh *et al*., 2015). This plant is widely cultivated commercially in India because it is well known as a root vegetable. The tubers are consumed as vegetables after boiling, roasting and frying (Nedunchezhiyan *et al*., 2002; Nedunchezhiyan, 2008). Elephant foot yam tubers have several medicinal properties and have been shown to be effective in the treatment of hemorrhoids, dysentery, asthma, inflammation of the lungs, vomiting and gastrointestinal disorders (Raghu *et al*., 1999). In Indonesia, this plant is starting to receive attention among farmers and the government because of its economic value. The area of elephant foot yam plantations in Indonesia in 2020 was 19,950 ha and in 2021 reached 47,461 ha spread over 15 provinces and is targeted to be a maximum of 100,000 ha in 2024 supported by processing industries and their markets (Ministry of Agriculture the Republic of Indonesia, 2021). Bali is one of provincies in Indonesia that grows elephant foot yam (Media Indonesia, 2021).

Several factors affect the growth and yield of elephant foot yam (Ravi *et al*., 2011; Suja *et al*., 2012). Lack of sufficient plant material (tubers or bulbil) of uniform size, poor tuber quality and presence of dormancy are the main constraints limiting elephant foot yam production (Bhagawan *et al*., 2008; Misra *et al*., 2001). Occurance of plant diseases both during seedling preparation and after transplanting in the field is also obviously affected the growth and yield of elephant foot yam. Diseases caused by fungi include: foot rot by the fungus *Rhizoctonia solani*, leaf blight by *Phytophthora colocasiae*, stem/tuber rot by *Phytium helicoides*, and *Slerotium rolfsii*. Bacterial diseases are wet rot caused by *Erwinia carotovora*, Konjac mosaic virus and Dasheen mosaic virus (DMV) (Saleh *et al*., 2015).

Foot rot disease caused by *R. solani*, is reported to be an important disease of *A*. campanulatus in southern India. Generally fungal infections are visible when the plant is two months old (Sivaprakazam *et al*., 1980). Symptoms of leaf blight caused by *Phytophthora colocasiae* generally occur in areas with relatively high temperatures (22-

23ºC), with relatively high relative humidity (85-100%) and high rainfall. Weather factors such as temperature, relative humidity, rainfall, total rainy days and wind speed jointly influence disease development, and disease severity is positively correlated with these factors (Singh *et al*., 2005).

Neck rot disease caused by the fungus *Sclerotium rolfsii* often infects plants that are often flooded and have poor drainage. This pathogen infection is also triggered by mechanical injury at the base of the stem. Symptoms of this disease are first characterized by brownish lesions on the neck which then spread to the entire pseudostem. At a heavy level of infection, the plant eventually dies (Jata *et al*., 2009).

In Indonesia, not much research has been done on pests and diseases in elephant foot yam, therefore this study was aimed to identify the main diseases appear during seedling preparation, as the healthy seedling is the key for the success of elephant foot yam cultivation.

2. Materials and Method

2.1. Disease Incidence Survey

The disease incidence survey was carried out in October 2022 in three locations which are porang breeding centers, namely Asah Duren Village and Bading Kayu Village, Jembrana Regency and Belalang Village, Tabanan Regency. Observations of disease incidence were carried out on three beds, consisting of 500 seedlings per bed. Observations were made on the type of disease and the incidence of disease for each disease based on the symptoms of the disease that appeared. Based on the type of disease, the number of seedlings showing disease symptoms per bed is then counted. The incidence of disease is calculated using the following formula:

$$DI = \frac{A}{500} \times 100\%$$

where:
DI= Disease incidence
A = number of seedlings showing symptoms of a particular type of disease
500= number of seedling per bed
For each type of disease, samples are taken of plant parts that show disease symptoms, then taken to the laboratory for observation.

2.2. Isolation and Identification of Pathogens

All plant parts showing disease symptom were washed in tape water to remove surface contamination, and then subjected to surface sterilization using 70% alcohol, and then rinsed with sterile distilled water to remove the remaining alcohol. The plant parts were then cut into small pieces, approximately 1x1 cm in size in a laminar air flow cabinet. The plant cuts were then transferred onto potato dextrose (PDA) medium and incubated in the dark at room temperature (28 + 2ºC) for 3 (three) days. The fungi appearred from the edge of plant cuts were isolated and transferred onto new PDA medium using sterile wire loop. All isolates of fungi obtained from this isolation were maintained in slant PDA medium before use for further observation. Shape and color of fungal colony were observed, and microscopic observation were done to determine the shape of conidia or spores using light microscope with 400 x magnification. The fungi were then purified and a single spore isolation was done prior to further use. Koch postulate procedure was done to ensure that the isolated fungi were the cause of diseases on elephant foot yam. All isolates were inoculated onto 15-days old seedlings of elephant foot yam. The development of symptom was observed seven days after incubation. Reisolation was done from infected plants to get pure isolates of pathogens. These isolates were then maintained on PDA slant medium before used.

Molecular identification was also done through analysis of 18S rRNA genes based on method developed by Photita *et al*. (2005).

The isolate of fungus (Pr1) the cause of neck rot disease was cultured on PDA medium for three days under room temperature (28±2ºC). DNA genomes of fungus was extracted by taking the hypae from the edge of colony and put into centrufuge tube and was suspended with 100 mL PrepMan Ultra reagent (PrepMan Ultra Protocol, Applied Biosystem, U.S.A.). Samples were then vortexed for 30 sec and put on heat block at 95ºC to 100ºC for 10 min and the was put under room temperature for 2 min. Samples were then centrifged at 10,000 rpm for 2 min and pellet containing DNA was taken and was used for further test (Cano *et al*., 2004). Ampliflication of 18S rRNA gene was done using PCR with two primers Internal Transkript Spacer (ITS) 1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS 4 (5-TCCTCCGCTTATTGATATGC-3). Reaction was done using Takara PCR thermal cycler Personal tool (Takara Bio, Otsu, Japan) with Ex Tag (Takara Bio, Otsu, Japan) under condition : pre-denaturation 94ºC (4 min) followed by 35 cycles denaturation at 94ºC (35 sec), annealing 52ºC (55 sec), elongation 72ºC (2 min) and post elongation 72ºC for 10 min (Nishizawa *et al*., 2010).

Nucleotide sequence was determined using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, C.A., U.S.A.) according to the manual of the equipment with PE Applied Biosystems Automated DNA Sequencer (model 3130xl, Applied Biosystems). Sequence of DNA double helix was assembled and analyzed using Genetyx (version 11.0) and Genetyx-ATSQ (version 4.0) software (Genetyx, Tokyo, Japan), in a sequential order and compared with the same DNA sequence that was accessed from DDBJ/EMBL/GenBank through NCBI BLAST program (Thompson *et al*., 1997).

Phylogeny analysis was done using MEGA 4.0 program (Kumar *et al*., 2001). Method of Neighbor Joining (NJ) was done with 1000x bootstrap with the following steps: (1). Seaching for similarities among sequences. Data was stored in otepad in a FASTA format, analyzed using Blast-WU facility available online in site of www.ebi.ac.uk/Clustalw. Based on similarity analysis can be identified if the level of similarity of 18S rRNA gene than 97% can be considered as a new species (Pangastuti, 2006). (2). Drawing up phylogenetic tree using MEGA program.

3. Results and Discussion

Based on the symptom**,** two types of diseases were found on seedlings of elephant foot yam namely neck rot and leaf blight diseases. The disease incidence of neck rot disease in three locations varied from 12.7% to 23.5% with an average of 18.7%, while the disease incidence for leaf blight disease varied from 2.5% to 3.8% with an average of 3.1%. The disease incidence in three locations of elephant foot yam seedlings production is presented in Table 1. Based on this data, neck rot disease is the main disease of elephant foot yam in Bali.

Table 1. Disease incidences in three locations of elephant foot yam seedling production in Bali in October 2022

No	Location	Disease incidence (%)	
		Neck rot disease	Leaf blight disease
1	Asah Duren Village	23.5	2.5
2	Bading Kayu Village	19.9	3.8
3	Belalang Village	12.7	3.0
Average		**18.7**	**3.1**

The symptom of the disease is shown as brownish lesions on the neck of the plant at the soil surface, and finally the plants were died as shown in Figure 1a. This symptom is similar to symptom of neck rot described by Jata *et al*., 2009. A fungus was isolated from the diseased plants and after passing the Postulate Koch procedure, the isolate Pr1 was proven to be the caused of the disease. This fungal isolate showed white colony on PDA medium and produced plenty of sclerotia, a resting spores with brown color as shown in Fug. 1b.

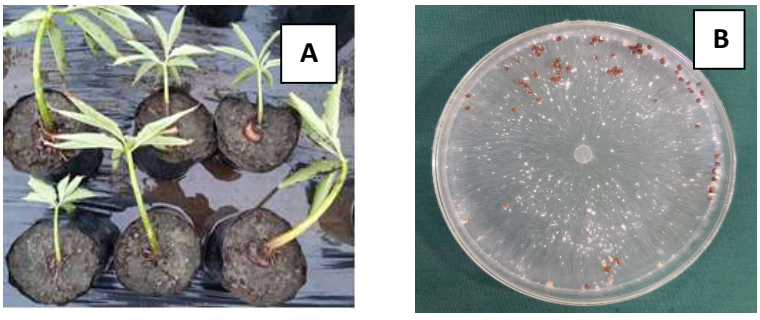

Figure 1. Symptom of neck rot disease on elephant foot yam seedlings (A) and the causal fungus showing white colony on PDA medium with plenty of brown color sclerotia (B).

Amplification of PCR product of sequence 18S rRNA gene 18 isolate Pr1 using two primers *viz*., Internal Transkript Spacer (ITS) 1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS 4 (5-TCCTCCGCTTATTGATATGC-3 resulted in DNA fragments of ± 682 bp (Fig. 2). The DNA fragment was then purified and subjected to sequencing to determine the species of fungus based on their similarities with other species of fungi that previously have been identified and deposited in GenBank. Based on sequence comparison with database of GenBank using

BLAST program, isolate Pr1 has close relationship several strains of *Athelia rolfsii* such as *A. rolfsii* CPC-23947, *A. rolfsii* BLH-Q, *A. rolfsii* IF 501, *A. rolfsii* YKY2020.02, *A. rolfsii* Scr, and *A. rolfsii* Ssr (Table 2 and Fig. 3).

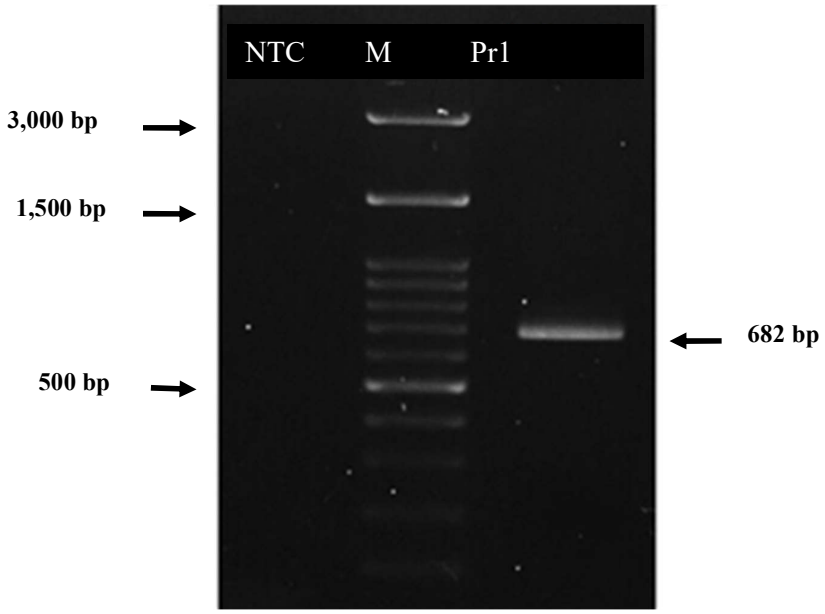

Figure 2. PCR product of 18S rRNA gene isolat Pr1 agarose gel.. M= 100 bp marker

Table 2. Sequence similarity of 18S rRNA gene of isolate Pr1 with several fungi deposited in GenBank

Isolate	Similarity (%)	Accession Number
Athelia rolfsii isolate CPC-23947	99.12	MK411221
Athelia rolfsii isolate BLH-Q	99.12	MG836252
Athelia rolfsii isolate IF 501	99.12	KY216142
Athelia rolfsii isolate YKY2020.02	99.12	OM647806
Athelia rolfsii isolate Scr	99.12	OM729593
Athelia rolfsii isolate Ssr	99.12	OM729592
Athelia rolfsii isolate W12	99.12	MW620998
Athelia rolfsii isolate W10	99.12	MW620996
Athelia rolfsii isolate W1	99.12	MW620994
Athelia rolfsii isolate W9	99.12	MW620995

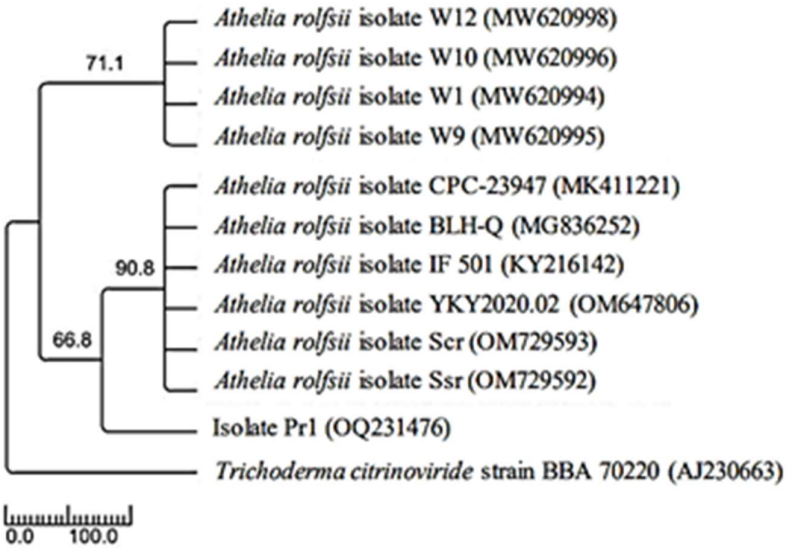

Figure 3. Phylogenetic relationship tree of isolate Pr1 based on 18S rRNA gene using Maximum Parsimony method

Several diseases have been reported occurred on elephant foot yam such as foot rot disease caused by the fungus *Rhizoctonia solani*, leaf blight by *Phytophthora colocasiae*, stem/tuber rot by *Phytium helicoides*, and neck rot disease caused by *Slerotium rolfsii*. Other diseases are also found in elephant foot yam namely wet rot caused by *Erwinia carotovora*, Konjac mosaic virus and Dasheen mosaic virus (DMV) (Saleh *et al*., 2015). In our study we found that neck rot disease is the main disease in seedling of elephant foot yam. The disease is caused by *Athelia rolfsii. Athelia rolfsii* is the sexual phase (teleomorph) of *Sclerotium rolfsii*. As the disease has economic importance, the control measures shliould be developed to produce healthy seedlings for transplanting of elephant foot yam. Ways to control this disease include planting truly healthy seeds, removing infected plants, improving drainage, using a botanical fungicide with neem leaf extract, or spraying with 0.2% Mancozeb fungicide (Jata *et al*., 2009). Gogoi *et al*. (2002) reported that a combination of tuber and soil treatment with *Trichoderma harzianum* reduced the incidence of *Sclerotium rolfsii* neck rot disease to 12.9%, followed by tuber and soil treatment with the fungicide Captan (0.2%) which was recorded at 14.8%, compared to 83, 3% in controls. The *T. harzianum* fungus will grow more quickly in the soil if it is treated as a soil treatment compared to tubers. Antagonistic fungal and chemical fungicide treatments significantly reduced the *R. rolfsii* population in the root area (Gogoi *et al*., 2002).

4. Conclusion

Two type of diseases were found in seedlings of elephant foot yam in Bali namely neck rot disease and leaf blight disease with disease incidence by 18.7% and 3.1% respectively. The neck rot disease severely infect the seedling and resulted in the plant death. The neck rot disease is caused by pathogenic fungus *Athelia rolfsii* Pr1.

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