

Investigation of Endophytic Fungi Towards Vascular Streik Dieback *Oncobasidium theobromae* on Seedling of Cocoa Plant

Amin NUR^{1*}

Salam MUSLIM²

ASMAN¹

RYAN¹

Rahim DANIAL¹

¹Department of Plant Protection, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia

²Department of Agricultural Social Economy, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia

*Corresponding author:

Email: nuramin_62@yahoo.com

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Abstract

Endophytic fungi are able to infect a wide range of host plant, which this is in some cases in plants associated to endophytes have shown increased resistance to plant pathogens, particularly fungal pathogens. The research aimed to investigate of endophytic fungi e.g *Aspergillus* sp, *Beauveria* sp, *Curvularia* sp, and *Trichoderma* sp isolated from healthy branches of cocoa plant towards vascular streak dieback disease on Seedling of cocoa plant in the field condition. All of fungal endophytes isolates showed the suppression of VSD and significantly different. Endophytic fungi *Curvularia* sp. and *Trichoderma* sp. was isolated from the offshoots of the seedling of cocoa plant after three months of inoculation. The fungal endophytes *Curvularia* sp. and *Trichoderma* sp. are endophytic fungus which provide potential biological control for Vascular Streik Dieback (VSD).

Keyword: Endophytic Fungi, Vascular Streak Dieback (VSD), *Oncobasidium theobromae*

INTRODUCTION

The main Indonesian cocoa producing region is the island of Sulawesi which accounts for around 75 percent of Indonesia's total cocoa production. As Indonesia's cocoa productivity per hectare has been lagging behind that of other cocoa-producing countries, the government started a five-year cocoa revitalization program in 2009 to boost production through intensification, rehabilitation and rejuvenation activities, covering a total area of 450 thousand hectares. Factors that are hampering progress in the cocoa industry are aging trees (planted in the 1980s), insufficient improved planting materials and little farm maintenance. More investment in this sector is needed to reach the government's one million tonnes annual production target by 2013-2014.

Main obstacle of cocoa growing is pest and disease infestations such as cocoa pod borer (CPB), Vascular streak disease of cocoa (VSD) and black pod disease, resulting in reduction of yield productivity about 40% (660 kg/ha/year) of 1100 kg/ha/year. Pest and disease infestation led to lose yield around 198,000 tonnes per year or it equaled to IDR 3.96 triliun per year. In addition, reduction of their infestations can cause poor bean quality so that cocoa bean export to United State of America faced potential loss around US\$ 301.5 per ton. VSD cocoa disease known new disease was found in 1985 in Kolaka Regency (Southeast Sulawesi) and in 2002 in Polmandistrict (West Sulawesi) and Pinrang (South Sulawesi) [15]. Although VSD is relatively new disease of cocoa, it spreads widely in the cocoa farmers in South Sulawesi. According to Data of State Crop Province of South Sulawesi that total cocoa areas infected VSD initially from about 7,000 ha in late 2003 to 20,607 ha end of July 2004 but it then doubled to 48,727 ha in 2009. Survey of Mars Incorporated in 2008 showed incidence of VSD disease in South Sulawesi around 21 – 68 % [14]. Moreover, in 2010 Directorate general of State Crop Protection claims that infestation of

VSD disease in 6 provinces in Indonesia last three years reached 212,132.92 ha. Based on above control of VSD cocoa disease requires seriously and rapidly.

There are several definitions of the term 'endophyte'. De Bary (1866) was the first to define organisms invading and residing within healthy host tissue as endophytes. More than a century later, Carroll [4] defined organisms causing asymptomatic infections within plant tissues as endophytes and excluded pathogenic fungi and special groups of mutualists such as mycorrhizal fungi. Petrini [12] expanded Carroll's definition to include all organisms which at certain times in their life inhabit plant organs without causing any harm. Endophytes have co-evolved for a very long period of time with their hosts and therefore usually show low virulence [18]. The behavior of fungal endophytes can range from mutualistic [22; 23] to pathogenic [20; 21], and endophytes can switch their behavior depending on environmental factors, described as the endophytic continuum [14]. Arnold et al. [1] could show that fungal leaf endophytes protect *Theobroma cacao* against *Phytophthora* diseases, and similarly Lee et al. [6] were able to show that the endophytic *Fusarium verticillioides* reduces disease severity of *Ustilago maydis* on maize. A number of authors have documented that the presence of endophytic fungi provide a protection of the plant hosts against insect herbivore [11], parasitic nematodes [7; 9], and plant pathogens [8].

Fungi ever isolated from cocoa plantation have potential as role of biological control agent for VSD cocoa disease. They also have identified morphologically and molecularly in genera: *Acremonium*, *Blastomyces*, *Botryspora*, *Cladosporium*, *Colletotrichum*, *Cordyceps*, *Diaporthe*, *Geotrichum*, *Gibberella*, *Gliocladium*, *Lasio-diplodia*, *Monilochoetes*, *Nectria*, *Pestalotiopsis*, *Phomopsis*, *Pleurotus*, *Pseudofusarium*, *Rhizopyrenis*, *Syncephalastrum*, *Trichoderma*, *Verticillium*, and *Xylaria* [16]

Relationship between endophytic fungi and the host is symbion mutualisms which both gain benefits each other to survive. Endophytic fungi gains substrate of nitrogen and carbohydrate from its host which substrate of host is poisonous compound and released afterwards it is consumed by endophytic fungi for life. The research aimed to investigate of endophytic fungi e.g *Aspergillus* sp, *Beauveria* sp, *Curvularia* sp, and *Trichoderma* sp isolated from healthy branches of cocoa plant against vascular streak dieback disease on Seedling of cocoa plant in the field condition.

MATERIALS AND METHODS

Source of Endophytic Fungi

Endophytic fungi *Aspergillus* sp, *Beauveria* sp, *Curvularia* sp and *Trichoderma* sp. was originally isolated from healthy branches of cocoa plant of local clone of South Sulawesi M.05 [10].

Production of Endophytic Fungi in Powder Form

Such endophytic fungi described as in point 2.1 propagated in rice medium. The rice medium that has been soaked for 3 hours put into a flask 100 grams, and autoclaved at 121 °C for 30 minutes and after which by using a corkborer (diam. 0.5 mm), five pieces of endophytic fungi were inoculated in once the fungi started growing, the flask were shaken to assure an even fungal growth. The grown fungi then incubated at 30 °C for 48 hours. The rice medium along with the fungi then blended to produce a powder form for further study.

Field Investigation of Endphytic Fungi against Vascular Streik Dieback (VSD)

The research was conducted in Soppeng district, South Sulawesi, Indonesia, which is endemic area of VSD cocoa planting. Seedling of cocoa plant of two months old were placed between two plants of infested VSD in the field. Application of endophytic fungi in powder form on seedling of cocoa made with a dose of 2 grams per seedling and sprayed directly on the seeds that had previously been given a gluten CMC with 0.7% concentration. The Research using a completely randomized design (CRD) with 5 treatments and 5 replications. Observations were made at 30, 60, 90 days after treatment application. The observation parameters are observed VSD symptoms arising in the leaves (ID). Measurement VSD disease at study sites was done by determining the intensity damaged (ID). Symptoms that appear in the formula is calculated based on the intensity damaged :

$$ID = \frac{\sum \text{Leave with Symptoms}}{\sum \text{Total Leaves}} \times 1$$

The Study On The Endophytisms of The Fungal Isolates

The study was carried out to observe whether the endophytic fungi applied might be able to penetrate into the offshoot of cocoa seedling. Isolation were made with the take of offshoot from the field, washed by using drop water, and cutted into small pieces (4mm). Every offshoot was taken 5 pieces, peeled and sectioned in order to result in 10 pieces. Those pieces were sterilized surface with NaOCl 2.5% for 3 minutes and alcohol 70% for 2 minutes, and then washed sterilized water for 1 minute. All pieces were laid into petridish covered by sterilized filter paper to dry out. They were then moved to Potato Dextrose Agar

(PDA). Afterwards, they were incubated in the room temperature for several days. From 3 to 14 days after incubation, the endophytic fungi were growing and isolated to pure culture in another PDA.

Data Analysis

Anova was also performed to determine the effects of endophytic fungi to intensity damaged. The percent data were arcsine-transformed before being subjected to Anova. When significant differences were detected, means were separated using Tukey's test at 5% probability level.

RESULT AND DISCUSSION

Result

Field Investigation of Endphytic Fungi Towards Vascular Streik Dieback (VSD)

The treatment of endophytic fungi *Curvularia* sp Isolate until 90 days after inoculation had no accident of VSD disease to compare with control 17 % and statistically significant differences (Table 1). Among treatment of endophytic fungi isolate is no statistically significant difference.

Table 1. Percent of Intensity Damaged of VSD Until 90 days after Inoculation with Endophytic Fungi Isolate on Seedling of Cocoa

Treatment	Intensity Damaged of VSD (%)		
	30 DAI	60 DAI	90 DAI
<i>Aspergillus</i> sp Isolate	0	0	4.7a
<i>Beauveria</i> sp Isolate	0	0	3.5a
<i>Curvularia</i> sp Isolate	0	0	0
<i>Trichoderma</i> sp Isolate	0	0	1.3a
Control	0	0	17.0b

Columns followed by different letters are significantly different from another according to Tukey's (Test ($P < 0.05$) $n = 5$).

DAI = Day After Inoculation With Endophytic Fungi Isolate

The Study On The Endophytisms Of The Fungal Isolates

There are only two isolates of endophytic fungi eg. *Curvularia* sp. and *Trichoderma* sp. which can be isolated again from the offshoots of the cocoa plant. Percent of endophytisms of *Curvularia* sp. and *Trichoderma* sp. is respectively 60 % and 40 % (Table 2).

Table 2. Percent of Endophytisms of The Isolate

Treatment	Endophytisms of The Isolate (%)
<i>Aspergillus</i> sp Isolate	0
<i>Beauveria</i> sp Isolate	0
<i>Curvularia</i> sp Isolate	60
<i>Curvularia</i> sp Isolate	40

DISCUSSION

Endophytic fungi *Aspergillus* sp, *Beauveria* sp, *Curvularia* sp. and *Trichoderma* sp showed a positive response against VSD (Table 1). The endophytic fungi protect plants from pathogen through the mechanisms of competition, induced resistance, antagonisms, and micoparasit [1]. *Trichoderma* sp can suppress pathogens through inhibition mechanism in the form of competition,

parasitism and antibiosis [2]. *Trichoderma* hyphae were able to coil around the hyphae of pathogens that inhibited growth of the pathogen, but it is also able to secrete the enzyme chitinase and β -1, 3 glucanase were able to remodel the cell wall of the pathogen. Moreover, *Trichoderma* is able to produce antibiotics 3-2 -hydroxypropyl-4-2-hexadienyl)-2-5 (5H). Furanone were able to inhibit the growth of pathogenic microbial spores and hyphae. Enzymes reported similar enzymes capable of acting as an elicitor in inducing plant resistance [2]. Endophytic fungi *Beauveria* sp and *Aspergillus* sp produce secondary metabolites such as chitinase enzymes that act as antibiosis and inhibit the development of fungal pathogen VSD in the plant tissue. St. Leger et al [19] reported that endophytic fungi *Beauveria* sp able to produce chitinase enzymes capable of degrading chitin. Bosah et al [3] also reported *Aspergillus* sp can produce the enzyme chitinase and β 1,3 glucanase (laminarinase) that has the ability to break down the cell wall components of pathogenic fungi such as chitin and β -1,3 glucan. Chitinase-producing fungi can inhibit even deadly pathogens because most of the structure-forming cell walls of fungi consist of chitin. This is in accordance with the opinion of Chernin et al [5] who suggested that chitinase-producing microorganisms can act as a biocontrol agent for various types of fungal plant diseases. Chitinase produced by micro-organisms can hydrolyze chitin structure, the main constituent of the cell wall compounds tube germination of spores and mycelia, so the fungus is unable to infect plants.

Percent of endophytisms of *Curvularia* sp. and *Trichoderma* sp. is respectively 60 % and 40 %. The same result reported Nur Amin et al [11] that *Trichoderma* sp. were able to penetrate in the host tissue. Similarly, in case of *Beauveria* sp. isolate until 90 days after inoculation was not found in the offshoot of seedling cocoa plant. Different result was reported by Posada et al [13] that *Beauveria bassiana* is an endophyte in cocoa pods after two or three months inoculation on the flowers. The ability of the associated fungus to penetrate in the offshoot proofed that they could grow in the intercellular of the offshoot and showed the ability of endophytisms and the offshoot of cocoa plant are conducive for the growth of naturally and inoculated fungal isolates consequently.

CONCLUSION

It can be concluded that, *Curvularia* sp. and *Trichoderma* sp are endophytic fungi which provide potential as biological control agent for vascular streak dieback disease of cocoa plant.

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