

Characterisation of *Colletotrichum siamense* TD1 causing anthracnose leaf spots of *Camellia tamdaoensis* Ninh et Hakoda at the Tam Dao National Park

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Abstract:

Camellia L. is a precious medicinal plant with 16 different species and high economic value. In Tam Dao National Park, Vinh Phuc, *Camellia* L. is rich in ingredients. Anthracnose is a severe disease caused by fungi on the leaves of *Camellia* L., however, research of the fungus causing anthracnose on leaves of *Camellia* L. in Vietnam remains limited. In this study, anthracnose-infected leaves of *Camellia tamdaoensis* Ninh et Hakoda in Tam Dao National Park were collected, and the causative agent was isolated. Eleven fungal strains that cause anthracnose were obtained through gene sequence identification of the internal transcribed spacer (*ITS*) region. One fungal strain was selected to isolate DNA and determine the gene sequence of the *ITS* region. Based on this *ITS* sequence, the causative agent on leaves of *C. tamdaoensis* Ninh et Hakoda was *Colletotrichum siamense* TD1. The *ITS* sequence was determined to be 522 bp in length, 52.30% of guanine-cytosine (GC) content, and was registered on GenBank with accession number OK560718. Pathogenicity tests were conducted to fulfill Koch's postulates. This is the first report of anthracnose leaf spots of *C. tamdaoensis* Ninh et Hakoda caused by *C. siamense* TD1 in Vietnam. The results of anthracnose fungi provide helpful information for disease management and are necessary for future studies on selecting disease-resistant varieties of *Camellia* L.

Keywords: anthracnose, *Camellia tamdaoensis* Ninh et Hakoda, *Colletotrichum siamense*, golden camellia, Tam Dao National Park.

Classification numbers: 3.1, 3.4

1. Introduction

Golden camellia belongs to the *Camellia* L. genus, Theaceae family. They are shrubs and small-sized trees that are naturally distributed in Vietnam. Its benefits are well-known in traditional medicine for improving human health. In Tam Dao, a large number of *Camellia* species were discovered in the National Park and natural zone [1]. Especially, *C. tamdaoensis* Ninh et Hakoda is one of the many unique plants scattered in the forest of this mountain. In recent years, anthracnose has become one of the most severe fungal diseases globally and severe anthracnose symptoms can be seen on the leaves of the *Camellia* genus. The first observed symptom appears as random chlorotic spots on leaves after which they coalesce and form large spots. The visual spots become dark brown or black depending on damage level [2]. In the past, morphological characters and host ranges were mainly used to classify the *Colletotrichum* species [3]. They were conidial, conidiophore, and acervuli morphology or sensitive to benomyl [4, 5]. However, it is challenging to identify taxonomies based on the morphological differences of *Colletotrichum* species because their morphological characteristics were strongly affected by both laboratory and

natural conditions. Currently, molecular tools give biologists many advantages to rapidly recognise *Colletotrichum* species at the genus level [3]. The nuclear *ITS* regions have been used to separate *Colletotrichum* species [4]. In this study, we aimed to collect a leaf with a disease related to anthracnose of *C. tamdaoensis* Ninh et Hakoda and describe the symptoms of this disease. Then, the fungal pathogen was isolated on a specific medium and molecular identification was performed based on sequencing the *ITS* sequence. Consequently, *C. tamdaoensis* Ninh et Hakoda was reinfected with the fungus to test pathogenicity ability.

2. Materials and methods

2.1. Collection of the disease leaf and describe the symptoms

C. tamdaoensis Ninh et Hakoda plant with anthracnose leaf spots were obtained from the Tam Dao National Park, Tam Dao district, Vinh Phuc province in May 2021. The symptoms of anthracnose disease were observed with an increase from small brown spots to black necrotic spots appearing on the leaf and stem. Diseased leaves of *C. tamdaoensis* Ninh et Hakoda were taken from these infected plants, packed in a

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