



Submissions for Diagnostic Protocols

I. General information

Submission number	2023-017
Title of Proposal	DP: <i>Colletotrichum kahawae</i> J.M. Waller & Bridge
Submitted by (Country or Organization)	IPPC Contracting Party
IPPC Official Contact Point or RPPO	China
Supported by	Technical Center of Ningbo Customs District, China

2. Contact information

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3. Summary of proposal

Summary of justification for the proposal	<p>Summary of justification for the proposal (provide an outline of the problem needing resolution in sufficient detail, 250 words max) <i>Colletotrichum</i> is one of the ten largest groups of plant pathogenic fungi in the world. The anthracnose disease of <i>Coffea arabica</i> berry caused by <i>C. kahawae</i> is a destructive disease, mainly affecting green berries and leaves, which can easily lead to fruit loss, resulting in up to 80% loss of coffee fruit yield, and it can also infect <i>Coffea canephora</i> and <i>Coffea liberica</i>. Currently, the pathogen is mainly distributed in many countries in Africa, Central America and Europe, etc. It has been included in the list of import quarantine by China and some countries in Asia and Latin America. In terms of morphological characteristics, <i>C. kahawae</i> is indistinguishable from closely related species, including <i>C. asianum</i>, <i>C. boninense</i>, <i>C. ciggaro</i>, <i>C. costaricense</i>, etc., some of which can also infect host plants of the genus <i>Coffea</i> and cause anthracnose. Therefore, it is necessary to establish a rapid and effective molecular detection method for <i>C. kahawae</i> in practice and applied research. Although molecular detection methods based on Real-time PCR and LAMP have been</p>
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	formulated in previous researches, the specific primers and probes used in these two technologies were not designed with the consideration of several closely related species of <i>C. kahawae</i> , and the detection time is long. Moreover, the biological activity of the species in the environment is not considered. Therefore, it is extremely urgent and practical to develop the method for detection and identification of <i>C. kahawae</i> by comparing genomes and transcripts, and combine various molecular technologies to formulate international standards for <i>C. kahawae</i> .
Proposed priority	I (high)
Comments	This proposal will be an important reference for relevant workers, and minimize the negative impact of <i>C. kahawae</i> as much as possible.

4. Literature review

Literature review	<p><i>Colletotrichum</i> comprises diverse plant pathogens, causing important diseases known as anthracnose (Cabral et al. 2020). Because of its phytopathological significance, the genus has been selected as one of the ten largest groups of plant pathogenic fungi in the world (Dean et al. 2012). <i>Colletotrichum</i> includes 16 species complexes and several singletons, with the <i>C. gloeosporioides</i> species complex having the highest number of species and the widest host range (Liu et al. 2022). Within <i>C. gloeosporioides</i> species complex, <i>C. kahawae</i> is a highly destructive pathogen that triggers Coffee Berry Disease (CBD) on <i>Coffea arabica</i> in Africa (Waller et al. 1993; Batista et al. 2017) where the infection causes up to 80% harvest loss without chemical control (Pires et al. 2016). Due to the potential threat it poses to the primary Arabica growing countries in America and Asia, this pathogen is classified as a quarantine concern, often referred to as a biological weapon (Batista et al. 2017, Vieira et al. 2018, A, Australia Group Common Control List Handbook 2020). In addition to <i>C. kahawae</i>, several other species in the <i>C. gloeosporioides</i> species complex are frequently isolated from ripe coffee berries, such as <i>C. fragariae</i>, <i>C. fructicola</i>, <i>C. asianum</i> and <i>C. siamense</i>, but they are incapable of causing CBD (Waller et al. 1993, Prihastuti et al. 2009). Moreover, some species are quite closely related to but are hardly distinguished from <i>C. kahawae</i>, such as <i>C. cigarro</i>, <i>C. fructivorum</i>, <i>C. jiangxiense</i>, <i>C. wuxiense</i> (Doyle et al. 2013, Liu et al. 2015, Wang et al. 2016). Therefore, accurate identification of <i>C. kahawae</i> and their differentiation from other closely related species is of crucial importance in quarantine work. Nevertheless, <i>C. kahawae</i> is morphologically indistinguishable from most of above closely related species in the <i>C. gloeosporioides</i> species complex (Weir et al. 2012), and is even indistinguishable from a group of <i>Colletotrichum</i> species for the six nuclear gene regions (<i>act</i>, <i>cal</i>, <i>chs1</i>, <i>gapdh</i>, <i>sod2</i>, <i>tub2</i>, <i>ITS</i>) that are usually employed for taxonomic purposes in this genus</p>
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	<p>(Weir et al. 2012). Therefore, the development of accurate and rapid detection technology applicable to the field is critical for quarantine efforts.</p> <p>Although researchers developed rapid assays for <i>C. kahawae</i> based on real-time PCR (Tao et al. 2012) and loop-mediated isothermal amplification (LAMP) (Tao and Cai 2013) in the early days, other close species that can infect coffee were not considered. In addition, after the publication of their research, taxonomists have discovered several new species closely related to <i>C. kahawae</i>, such as <i>C. cigarro</i>, but it is still unknown whether the existing specific primers can distinguish these species. Based on literature review, commonly used fragment sequences cannot effectively distinguish <i>C. kahawae</i> and its relatives. Therefore, it is necessary to re-evaluate the effectiveness of existing <i>C. kahawae</i>-specific primers. In order to more accurately identify the biological weapon <i>C. kahawae</i> and improve the detection efficiency and accuracy of the quarantine department, this project plans to find differential genes through genome and transcriptome comparison, design specific primers, and develop rapid detection methods.</p> <p>References</p> <p>Australia Group Common Control List Handbook—Volume II: Biological Weapons-Related Common Control Lists. Available online: https://australiagroup.net/en/documents/Australia-Group-Common-Control-ListHandbook-Volume-II.pdf (accessed on 6 March 2020).</p> <p>Batista, D.; Silva, D.N.; Vieira, A.; et al. (2017). Legitimacy and implications of reducing <i>Colletotrichum kahawae</i> to subspecies in plant pathology. <i>Front. Plant Sci.</i> 7, 2051.</p> <p>Cabral, A., Azinheira, H. G., Talhinhos, P., et al. (2020). Pathological, morphological, cytogenomic, biochemical and molecular data support the distinction between <i>Colletotrichum cigarro</i> comb. et stat. nov. and <i>Colletotrichum kahawae</i>. <i>Plants</i>, 9(4), 502.</p>
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5. Criteria for prioritization of Diagnostic Protocols

Criteria	Information provided by Submitter
I. Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology)	<p>Due to the potential threat <i>Colletotrichum kahawae</i> poses to the primary Arabica growing countries in America and Asia, this pathogen is classified as a quarantine concern, often referred to as a biological weapon. A group of <i>Colletotrichum</i> strains/species from multiple hosts and diverse geographic origins were shown to be indistinguishable from <i>C. kahawae</i> for six nuclear gene regions (<i>act</i>, <i>cal</i>, <i>chs1</i>, <i>gapdh</i>, <i>sod2</i>, <i>tub2</i>, <i>ITS</i>) that are usually employed for taxonomic and diagnostic purposes, in spite of a clear differentiation provided by the mating type gene <i>mat1-2-1</i> (<i>mat1-2-1</i>), a fragment of DNA lyase <i>Apn2</i> (<i>apn25L</i>), and glutamine synthetase (<i>gs</i>). However, the success rate of amplification of these fragments is not 100%, and it takes longer time to compare the sequence fragments than on-site detection technology.</p>

<p>2. The relevance of the diagnosis to the protection of plants including measures to limit the impact of the pest.</p>	<p>Coffea arabica is the most important coffee variety, accounting for about 3/4 of the world's total coffee production. It is mainly grown in Latin American countries, and some are grown in Indonesia and the Pacific Islands. Colletotrichum kahawae is a highly destructive pathogen that triggers Coffee Berry Disease (CBD) on Coffea arabica and is currently restricted to Africa where the infection causes up to 80% harvest loss without chemical control. Moreover, it has been referred to as a biological weapon to the Arabica growing countries in America and Asia. Therefore, diagnosis and quarantine treatment of C. kahawae are of great significance for the protection of coffee cultivation in the world.</p>
<p>3. Importance of the plants protected on the global level (e.g. relevant to many countries or of major importance to a few countries).</p>	<p>It is relevant to many countries in America, Asia and Pacific islands where growing Coffee arabica.</p>
<p>4. Volume / importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).</p>	<p>World coffee production for 2023/24 is forecast 4.3 million bags (60 kilograms) higher than the previous year to 174.3 million. Coffee is commercially produced in more than 50 countries, and the world drinks upwards of 3 three billion cups a day. The annual income of the coffee sector is estimated to exceed \$200 billion.</p>
<p>5. Other criteria for topics as determined by CPM that are relevant to determining priorities</p>	<p>Coffee is one of the world's most popular beverages today. Clear identification of the pathogen that need to be resolved through the development of the standard. Therefore, there is a urgent need for the standard for C. kahawae.</p>
<p>6. The balance between pests of importance in different climatic zones (temperate, tropics etc) and commodity classes.</p>	<p>Colletotrichum kahawae that causes Coffee Berry Disease is an aggressive and specialized fungal pathogen affects coffee production with great damage on the economic parts of green coffee berries (Coffea arabica L) in Africa. C. kahawae attacks coffee berries at all stages of development from flowering to ripening. The considerable coffee loss occurs following the infection of the pathogen.</p>
<p>7. Number of labs undertaking the diagnosis.</p>	<p>As far as we know, there are two labs are undertaking the diagnostic method research, namely, State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, and Technical Center of Ningbo Customs District P. R. China.</p>
<p>8. Feasibility of production of a protocol, including availability of knowledge and expertise.</p>	<p>Several experts, including the submitter, pay long-term attention to this</p>