First report of *Phyllosticta* spp. associated with banana freckle disease in southern Lao PDR

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Abstract

A survey of small-holder banana farms was undertaken in February/March 2019 in Savannakhet, Salavan, Sekong and Champasak provinces in southern Lao People's Democratic Republic (Lao PDR) to establish which banana leaf pathogens were present. Twenty-five farms were visited, seven different cultivars were assessed, and 13 leaf samples with characteristic symptoms of banana freckle disease collected. *Phyllosticta musarum* was detected on the variety 'Kuay Nam' in each of the four provinces surveyed. *Phyllosticta cavendishii* was detected in Salavan, Sekong and Champasak. *Phyllosticta musaechinensis* was recorded from Champasak. This is the first time these three species have been recorded associated with banana freckle disease in the Lao PDR.

Keywords Laos · Southeast Asia · *Musa* · Leaf disease · Survey · *Guignardia musae*

Banana freckle disease is caused by species of *Phyllosticta*, including *P. musarum* (reported from Malaysia, India and Thailand), *P. maculata* (American Samoa, Australia, Indonesia, Malaysia and Papua New Guinea), and *P. cavendishii* (Taiwan, Indonesia, Vietnam, Timor Leste, Malaysia, United States of America, Federated States of Micronesia, Tonga and Australia) (Wong et al. 2012; McMaster et al. 2020). There is some evidence of host specificity of *Phyllosticta* species on banana, with *P. cavendishii*, thus far the only *Phyllosticta* species associated with disease symptoms on 'Cavendish' banana (Wong et al. 2012). *P. musaechinensis* has been reported associated with banana freckle disease in

China, but Koch's postulates have not been completed, with the authors stating it is a weak pathogen of banana leaves (Wu et al. 2014).

Banana freckle disease affects banana leaves and fruit. On leaves the symptoms are red/brown spots 1–4 mm in diameter. The smaller lesions may give the leaf a sooty appearance, larger lesions may be associated with lesions with grey centres and green halos. The lesions may coalesce in clumps or streaks and are rough to the touch (Punithalingam and Holliday 1975; Wong et al. 2012) which are a useful diagnostic feature (Ploetz et al. 2003). On fruit, lesions are generally 2 mm in diameter with small green halos (Punithalingam and Holliday 1975). On fruit the presence of the raised lesions as a diagnostic feature is present, but should not be confused with raised lesions caused by banana flower thrip oviposition in countries where this pest is present.

Bananas are a staple food and fruit crop in the Lao People's Democratic Republic (Lao PDR) providing much needed nutrition and can be sold for cash, they are also culturally important (Vansilalom 2016). At the same time exports from foreign owned banana plantations have been growing rapidly making it the largest agricultural export for the country (Food and Agriculture Organization of the United Nations 2021). There is very little information on the pathogens of banana in Lao PDR (Vansilalom 2016). Therefore, our aim was to identify the pathogens present on

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Table 1 BRIP Herbarium number, GenBank accession number, location of collection, cultivar and identification

BRIP number	GenBank accession number	Location	GPS way point	Cultivar if known	Molecular identification of pathogen
72558	MZ404693	Sepon, Savannakhet	16.6625, 106.3034	Kuay Nam	<i>Phyllosticta musarum</i>
72559	MZ404694	Sepon, Savannakhet	16.5973, 106.4225	Kuay Nam	<i>Phyllosticta musarum</i>
72,570	MZ404703	Savannakhet, Savannakhet	16.5389, 104.8467	Kuay Nam	<i>Phyllosticta musarum</i>
72,555	MZ404691	Pakse, Champasak	15.0614, 105.8278		<i>Phyllosticta musaechinensis</i>
72,556	MZ404692	Paksong, Champasak	15.167, 106.0368		<i>Phyllosticta cavendishii</i>
72568	MZ404701	Don Yang, Champasak	14.1293, 105.8604	Kuay Nam	<i>Phyllosticta musarum</i>
72569	MZ404702	Ban Bangliang, Champasak	14.9808, 105.9133	Kuay Nam	<i>Phyllosticta musarum</i>
72562	MZ404695	Thateng, Sekong	15.5062, 106.3456	Kuay Ngao	<i>Phyllosticta cavendishii</i>
72,566	MZ404699	Thateng, Sekong	15.4956, 106.3444	Kuay Nam	<i>Phyllosticta musarum</i>
72567	MZ404700	Thateng, Sekong	15.4344, 106.3871	Kuay Ngao	<i>Phyllosticta cavendishii</i>
72,563	MZ404696	Tad Lo, Salavan	15.542, 106.2723	Kuay Nam	<i>Phyllosticta musarum</i>
72,564	MZ404697	Tad Lo, Salavan	15.495, 106.2434	Kuay Nam	<i>Phyllosticta musarum</i>
72565	MZ404698	Tad Lo, Salavan	15.495, 106.2434		<i>Phyllosticta cavendishii</i>

banana in the provinces of Savannakhet, Salavan, Sekong and Champasak. This report is focussed on our findings in respect to pathogens associated with symptoms typical of banana freckle disease.

Visits to small-holder farms in each of the four provinces were conducted in the dry season (February and March 2019) to facilitate access to farms. Farms were surveyed on foot (observing appropriate biosecurity processes), any symptoms of leaf diseases were noted and photographed, and GPS way points recorded. Leaves with characteristic freckle lesions were collected, placed into paper envelopes and processed later the same day. The banana variety sampled was determined visually by an experienced local officer.

Across the four provinces twenty-five sites were visited, seven different banana varieties surveyed and thirteen plants with characteristic banana freckle disease symptoms were visually identified and samples collected (Table 1, Fig. 1).

Due to the very slow growing nature of *Phyllosticta* spp. (Wong et al. 2012) and the limited time in country it was not possible to isolate pure cultures of putative pathogens. Consequently, segments of leaves showing symptoms of banana freckle disease were cut into squares approximately 30 × 30 mm and dried over calcium chloride for transport

to Australia. On entry into Australia the dried leaf samples were gamma irradiated as per requirements of the import permit and submitted to the Queensland Plant Pathology Herbarium (BRIP) as voucher specimens. In addition, sections of adjacent leaf tissue of the voucher specimens with characteristic symptoms were photographed, wiped with 70% ethanol using sterile wipes, cut into narrow strips using sterile instruments (approx. 4 × 35 mm) and placed into 80% ethanol for transport to Australia for DNA extraction as per requirements of the import permit.

On return to Australia the leaf samples preserved in 80% ethanol were drained of the ethanol and freeze dried. DNA was extracted from these samples using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. To confirm the presence of *Phyllosticta* spp. a PCR using primers GmF1/GmR2 (Wong et al. 2012), which amplify part of the internal transcribed spacer (ITS) regions ITS1 and ITS2 and the 5.8S rRNA gene, was conducted on all samples. The GmF1/GmR2 PCR products were sent for purification and direct sequencing to Macrogen Inc. (Seoul, South Korea). Sequences were aligned using Geneious 10 (<https://www.geneious.com>).

Fig. 1 Characteristic symptoms of banana freckle disease associated with **a** *Phyllosticta musarum* on 'Kuay Nam', **b** *P. cavendishii* on 'Kuay Ngao' and **c** *P. musaechinensis* on unknown cultivar. Scale bar 10 mm

Fig. 2 Molecular phylogenetic tree based on partial ITS sequence. Green dots indicate sequences generated in this study. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model in MEGA7. The tree

with the highest log likelihood is presented. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site

Identification of the samples was undertaken by comparing our sequences to those obtained by Wong et al. (2012), as well as those generated by Wu et al. (2014) and McMaster et al. (2020) using MEGA7 analytical software (Kumar et al. 2016). Forty-five sequences were included in the analysis which covered a length of 391 nucleotides. The use of sequence data

of the partial ITS is appropriate for identification of banana *Phyllosticta* species as Wong et al. (2012) provided a robust framework for comparison.

Phyllosticta musarum was commonly identified, *P. cavendishii* less often, *P. musaechinensis* was associated with one sample, and *P. maculata* was not detected (Fig. 2). *P.*

musaechinensis has only been recorded once before by Wu et al. (2014) who refer to it as a weak pathogen. ‘Kuay Nam’ (Pisang Awak subgroup, ABB) was the most commonly grown variety and *Phyllosticta musarum* was detected on this variety in each of the four provinces surveyed. *P. cavendishii* was detected twice on ‘Kuay Ngao’ (Cavendish subgroup, AAA), but was not detected on ‘Kuay Nam’, and it would be interesting to know if this could be attributed to a sampling artefact, or if ‘Kuay Nam’ is resistant to *P. cavendishii*.

As far as we are aware, this is the first report of *Phyllosticta* spp. associated with banana in Lao PDR. The current control measure for this disease, and other banana leaf diseases, in southern Lao is occasional removal of affected leaves and leaf parts. It is unlikely that there will be a practice change by small-holder growers to manage the disease as, even with the presence of leaf diseases, local supply chains are short and bananas are a valuable, relatively low-input, poverty alleviation crop.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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